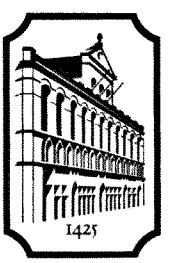
ACTA BIOMEDICA LOVANIENSIA 136

Hilde CAULIER

AN ANIMAL EXPERIMENTAL STUDY TO IMPROVE THE SUCCESS RATE OF ORAL IMPLANTS IN BONE OF LOW DENSITY:

The Influence of Ca-P Coatings



LEUVEN UNIVERSITY PRESS

ISBN 90 6186 762 2
D/1996/1869/40

AN ANIMAL EXPERIMENTAL STUDY TO IMPROVE THE SUCCESS RATE OF ORAL IMPLANTS IN BONE OF LOW DENSITY:

The Influence of Ca-P Coatings

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

PROEFSCHRIFT
ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 17 september 1996
des namiddags om 1.30 uur precies

door

Hilde CAULIER

geboren in 1968 te Herk-de-Stad (België)

LEUVEN UNIVERSITY PRESS

Promotores:	Prof. Dr. J.A. Jansen Prof. Dr. I. Naert, K.U. Leuven, België Prof. Dr. W. Kalk
Manuscriptcommissie:	Prof. Dr. H. Renggli, voorzitter Prof. Dr. K. de Groot, RU Leiden Prof. Dr. D. van Steenberghe, K.U. Leuven, België Prof. Dr. R. Veth Prof. Dr. M.A.J. van Waas, ACTA Amsterdam

Part of the research of this thesis was financially supported by the Stichting voor Technische Wetenschappen (STW), grant nr. NTH 33.2942

The printing of this thesis was financially supported by contributions from Nobel Biocare Benelux B.V.

This thesis is based on the following publications:

J.A. Jansen, **H. Caulier**, M.A. van t'Hof, I. Naert, "The evaluation of Ca-P coatings in the animal experiment: the importance of the study design." (submitted).

H. Caulier, J.P.C.M. van der Waerden, Y.C.G.J. Paquay, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "Effect of calcium phosphate (Ca-P) coatings on trabecular bone response: A histological study.", J. Biomed. Mater. Res., 29, 1061-1069, 1995.

F.G.A. Corten, **H. Caulier**, J.P.C.M. van der Waerden, W. Kalk, F.H.M. Corstens, J.A. Jansen, "The assessment of bone surrounding implants in goats: *ex vivo* measurements by Dual X-ray Absorptiometry." (submitted).

H. Caulier, T. Hayakawa, I. Naert, J.P.C.M. van der Waerden, J.G.C. Wolke, J.A. Jansen, "An animal study on the bone behaviour of Ca-P coated implants: the influence of implant location." (submitted).

H. Caulier, J.P.C.M. van der Waerden, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "A histological and histomorphometrical evaluation of the application of screw-designed calciumphosphate (Ca-P) coated implants in the cancellous maxillary bone of the goat." J. Biomed. Mater. Res., 1996 (accepted).

H. Caulier, I. Naert, W. Kalk, J.A. Jansen, "The relationship of some histological parameters, radiographic evaluations and Periotest® measurements of oral implants; an experimental animal study." (submitted).

H. Caulier, S. Vercaigne, I. Naert, J.P.C.M. van der Waerden, J.G.C. Wolke, W. Kalk, J.A. Jansen, "The effect of Ca-P plasma-sprayed coatings on the initial bone healing of oral implants; an experimental study in the goat.", J. Biomed. Mater. Res., 1996 (accepted).

CONTENTS

Chapter 1

GENERAL INTRODUCTION

1.1. INTRODUCTION

The ageing of the population in our welfare state is giving rise to increasing medical problems. Besides the increased occurrence of general medical problems, this has also resulted in more patients suffering from the loss of their teeth and due to this from the loss of jaw bone. Although, many patients with alveolar bone loss can be treated with conventional prosthetic appliances, an ever growing group has problems with the retention or acceptance of a removable prosthesis. Occasionally, a helpful short-term solution for these patients can be found in surgical procedures like augmentation of the jaw bone to improve the retention and stability of their dentures. Fortunately, since 1975 enormous progress has been made in implant dentistry. Therefore, currently, the use of oral implants can be considered a safe and satisfactory treatment concept for the support of a dental prosthesis. Already several clinical studies have shown that oral implants can be maintained without problems for long periods of time [1-6]. This clinical success is determined by a series of factors, which are mostly related to the quality and quantity of the remaining jaw bone. In this respect, the reactions that occur at the bone-implant interface are important. In the next section, the healing response of bone around implants and some of the factors that influence this reaction will be discussed.

1.2. INTERFACE BETWEEN BONE AND IMPLANT

The long-term clinical success of oral implants is based on the presence and maintenance of direct bone-implant contact. Knowledge of the histological and bone-healing processes is required to understand and discuss how bone remodels to an oral implant.

1.2.1. Histological background

The bone matrix is a highly ordered composite consisting of 22 % organic

matrix, 70 % inorganic mineral, and 8 % water (weight %) [7, 8]. The volume fractions of the organic matrix and inorganic mineral are about the same as the mineral has a density of 3.156 g/cm^3 and the organic matrix of about 1 g/cm^3. The organic phase consists mainly of collagen, which is laid down in the form of fibbers. In addition to the collagen, glycoproteins and proteoglycans are also present. The principal constituents of bone mineral, the inorganic phase, are calcium-phosphate and calcium carbonate with lesser quantities of sodium, magnesium, and fluoride. The mineral components consist mainly of a mixture of needle-like hydroxyapatite crystals, oriented alongside the collagenous fibbers, and amorphous calcium-phosphate. The unit cell of hydroxyapatite is described by the formula $Ca_{10}(PO_4)_6(OH)_2$.

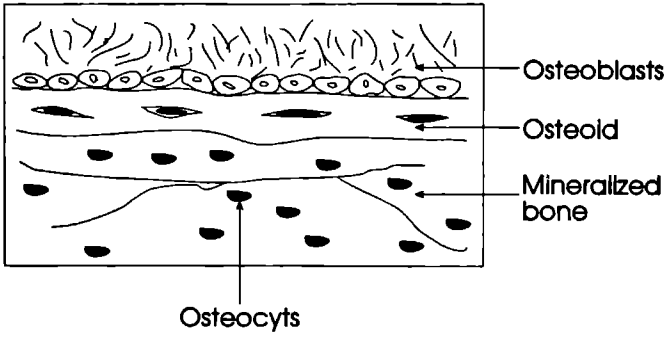


Figure 1.1: Schematic drawing of osteoblasts arranged in an epitheloideal fashion that lines the bone tissue and separates it from the remaining tissue.

The origin, function, and appearance of the three types of cells present in the bone differs. Osteoblasts originate from stromal stem cells and are responsible for the formation of bone during growth, remodelling, and repair processes. They are arranged in an epitheloideal fashion, i.e., as a continuous layer of cells that lines the bone tissue and separates it from the remaining tissue (Figure 1.1). The cells can be active or inactive, which is histologically visible by the shape and contents of the cell. Osteoblasts may become embedded in the bone

matrix as these cells not only deposit the organic matrix between the osteoblasts and the already existing bone but also between the osteoblasts themselves.
After osteoblasts are incorporated by bone matrix, they are called osteocytes. Osteocytes are completely surrounded by mineralised bone matrix with the exception of a 1-2 mm wide space which forms the osteocyte lacuna. Osteocytes facilitate the exchange of oxygen, nutritional factors, and metabolites with the blood by canaliculi connecting to the neighbouring osteocytes (Figure 1.2).

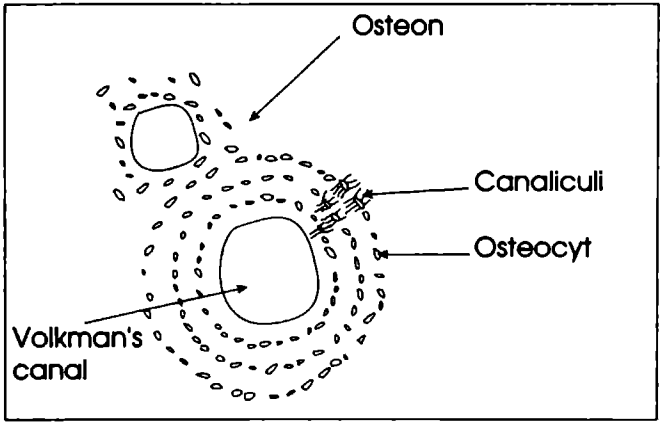


Figure 1.2: Schematic drawing of osteocytes connected to neighbouring osteocytes by canaliculi.

Osteoclasts are multinucleated giant cells that occur either single or in small groups on the inner surfaces of trabecular and compact bones. They originate from monocytes, blood-borne cells that are originally formed from premonocytes in the marrow. The cells are able to resorb bone and are active throughout the entire lifetime.
On the macroscopical level, there are two types of bone tissue: cortical or compact and cancellous or trabecular bone. The classification of bone tissue as cortical or cancellous is based on bone porosity, which is the proportion of the volume occupied by non-mineralised tissue. Cortical bone has a porosity of

approximately 5 % to 30 %; cancellous bone porosity may range from approximately 30 % to more than 90 %. Therefore, cortical bone has a slightly higher true density than cancellous bone due to the replacement of water by mineral constituents [9]. Further, cortical bone contains basic structural units called osteons, which are cylindrically shaped and have a central vascular canal called the Volkman's canal.

Microscopically, cancellous bone is a 3-dimensional network of bony plates and columns. The trabeculae divide the interior volume of bone into intercommunicating pores of different dimensions, thus producing a structure of variable porosity and apparent density. There are no blood vessels within the trabeculae, but they are present immediately adjacent to the trabeculae and in the space between the trabeculae. A dense compact shell of cortical bone encloses the trabecular bone.

Two additional types of bone can be observed based on the orientation of the collagen fibbers, i.e. woven and lamellar. The collagen fibbers in woven bone run in three directions. In lamellar bone the collagen fibbers show a regular orientation in the form of plates. Woven bone is an immature form of bone that is replaced by lamellar bone during normal maturation and that is formed directly after fractures or tooth extraction. Subsequently, it is replaced by lamellar bone [10-12].

1.2.2. Bone healing

Bone healing after trauma or fracture can be divided into three sequential phases:

* *Inflammatory phase:* On both sites of the line of damaged or fractured bone, blood from the vessels within the fracture site fills the tissue spaces and forms a clot. Following accumulation and coagulation of the clot, an acute inflammatory response occurs within the bone defect with vasodilatation and exudate of plasma and leukocytes followed by macrophages.
* *Repair phase:* In this phase the blood clot is invaded by fibrovascular tissue (revascularization) that replaces the clot. Devitalised necrotic bone is

resorbed by osteoclasts recruited through the trauma. Osteocytes do not occur in the repair process, but mesenchymal cells from periosteum and endosteum differentiate into bone-forming or cartilage-forming cells, depending on the environment at the site of the bone defect. This fibrous or cartilaginous callus envelops the bone ends and increases the stability of fracture fragments. The callus is subsequently replaced and woven bone is formed by intramembranous ossification (bone formation through the differentiation of mesenchymal cells into osteoblasts), or endochondral ossification (differentiation of mesenchymal cells into chondroblasts, which in turn ossify) [13].

* *Remodelling phase:* Following woven-bone formation, an internal reorganisation (remodelling) starts by which new lamellar bone is formed with a functional orientation. It is important to emphasise that bone modelling and remodelling (renewal and substitution) are continuous ongoing processes independent of age or functional condition. Therefore, two types of bone remodelling can be discerned:
  1. systemic, which is regulated by hormones
  2. local, which is activated by trauma and local tissue factors

1.2.3. Healing response of bone to oral implants

Directly after insertion, an oral implant is surrounded by a zone of necrotic bone [14]. During the initial stages of bone healing this devitalised bone is removed by osteoclastic activity. The osteoblasts originate from the bony environment. In addition, similar to fracture healing, a callus is formed that bridges the defect between the wound margins and the implant surface. The initial callus will mature under the proper conditions [15]. This will result in a strong compact bone interface that can resist the forces of mastication. Regular and continuous remodelling of the bone is required to maintain the bony fixation.

As will be discussed in detail in section 1.3 below, the healing process can be disturbed. Then mature bone deposition will not occur, but an intervening fibrous tissue interface will develop. The presence of such a capsule will predictably not

result in the long-term fixation of the implant. Eventually, the implant may fail [1, 16].

1.3. FACTORS INFLUENCING IMPLANT SUCCESS

The following factors are considered to be relevant for the successful remodelling of the bone around oral implants:

1. the implantation site
2. the implant surgery
3. the biomechanical conditions
4. the implant material
5. the implant surface properties

1.3.1. The implantation site

The implantation site and the local bone conditions are of great importance for a reliable implant-bone anchorage [17]. The significance of these factors can be indirectly derived from the observed difference in survival rate between implants inserted into the maxilla and into the mandible. For example, a recent study performed in the Netherlands has demonstrated that the three-year implant-survival rate of implants inserted in the mandible for overdentures is 96.7 % while the survival rate of the maxilla is 70.6 % [18, 19]. It has been suggested that limited bone volume and lower bone density in the maxilla are responsible for this difference.

The presence of this low-density bone is thought to be the major reason for the poor performance of maxillary implants [21 (see chapter 5), 22] compared with mandibular implants.

To categorise the condition of the residual jaw bone and to identify the problems, Lekholm and Zarb [23] have proposed a classification system. They used the amount of the remaining compact and dense trabecular bone as a

grading scale for bone quality. According to this scale, the maxilla consists mostly of cancellous bone or Type III and Type IV bone.

Despite this assumed relationship between the state of the host bed and implant success, it must be noted that bone-metabolic diseases (osteorarthritis, osteoporosis) and hormonal imbalances (prolonged steroid treatment, diabetes) are not considered to be an absolute contraindication for the insertion of oral implants as long as the calcium balance and other critical aspects of the metabolic disease process are controlled. However, installation of implants is contraindicated when the patient is being treated with radiotherapy [24, 25].

1.3.2. Implant surgery

Oral implants have to be installed according to a surgical protocol that limits trauma. For example, extensive stripping of the periosteum will compromise the vitality of the osteoprogenitor cells (bone cells that line the bone surface and that have the capacity to divide and proliferate). The resulting reduced osteogenic reaction will limit the initial healing response [15]. Moreover, too much frictional heat generated by drilling procedures will give rise to a zone of devitalised bone that can endanger the formation of a viable bone-implant interface [26-28]. It is known, that the extent of this necrotic zone varies exponentially with the magnitude of the heat generated by surgical trauma [29]. For example, histomorphometrical experiments [30, 31] have shown that, after implant installation, bone remodelling processes take place in a zone of 0.5-1.0 mm around the implant. This damage is also influenced by the bone quality of the implant area. Well-vascularized cancellous bone will dissipate the heat faster and has a greater capacity for regeneration than poorly vascularized compact bone [32].

To reduce the risk of surgical trauma, the surgeon should use a graded series of drills. The drilling should be performed (1) intermittently at low speed and low pressure, so that the cut bone can escape and access created for the irrigation fluid [26, 33], and (2) under constant and profuse internal and/or external cooling with a saline solution [32, 34].

The fit of the implant in the drilled implant bed can also influence the final bone-implant contact. Carlsson *et al.* [35] found that gaps of 0.35 mm and 0.85 mm around stable, smooth titanium implants were not bridged by bone. They concluded that the critical gap between the bone and a cylindrical implant, that prevents direct bone apposition on the implants is close to zero. Søballe *et al.* [36] investigated the influence of HA-coating on the bone response to press-fit implants and non-interference fit inserted implants four weeks after implantation into the femora of dogs. The non-interference fit implants were surrounded by a gap of 1 mm. Histological evaluation demonstrated a significant increase in the amount of bone in direct contact to HA-coated implants compared with non-coated gap and press-fit inserted implants. Thus, he concluded that HA-coating eliminates the influence of surgical fit on the skeletal fixation of an implant. In contrast, Ducheyne [37], Oonishi [38], and Jansen [39] suggest that a gap may be present around an implant, although it should be noted, that some of them created two to three times smaller gaps around the implants than did Carlsson and Søballe. For example, Jansen [39] inserted non-coated and HA-coated titanium implants with gaps of 0.1 and 0.25 mm into the tibia of rabbits. After three months of implantation, the interfacial bone reaction appeared to be the same for both coated and non-coated implants with the various degrees of surgical fit.

1.3.3. Biomechanical implant conditions

Oral implants are exposed to intraoral forces and movements, regardless of their structure or design. The load exerted by these forces will be transmitted to the interfacial tissues. Consequently, the final incorporation of an implant in a dynamic structure like bone is only possible when the implant is in mechanical equilibrium with its host. For a better understanding of the underlying fundamental concepts and principles, some knowledge of bone biomechanics is required. The textbooks of Frost [40], Block [41] and Buser *et al.* [42] have been used as reference material in the preparation of this section.

Bone responds to loading. This adaptive behaviour was already recognised

more than a century ago by Wolff [43] and Roux [44]. Their theories are referred to as the law of orthogonality and law of transformation of bone. These laws are based on the assumption that (1) trabecular bone alignment is due to functional forces and that (2) tensile or pressure forces stimulate bone formation. As a result, a piece of bone will be maintained where required and resorbed where superfluous. In terms of bone remodelling activity this means that changes in functional forces like those occurring after implant installation will result in measurable changes in the bone architecture. This phenomenon also implies that the survival time of implants will increase when the bone turnover rate can be modified in such a way that the integrity of the implant-bone interface is maintained or even improved. In this context, Jensen [45] reported the hypertrophic bone response to stress imposed by titanium implants on the bone. In his model he used the Carter hypothesis [46] on the relationship between bone strain and bone volume change (Figure 1.3). According to this hypothesis, a hypertrophic response develops at high strain rates and bone atrophy occurs at low strain rates. In his opinion, a hypertrophic response is a sign of implant functionality. These considerations also form the argument for an intervening non-loaded healing period after installation of the oral implants. In non-traumatised bone, microdamage in response to normal stress and strain patterns causes an extensive cellular repair response. Unfortunately, in traumatised bone, such as caused by drilling, this repair mechanism is impaired. When an implant is loaded under these conditions, the development of a bony fixation will be prevented.

Moreover, a mismatch in the mechanical properties between the implant and surrounding bone will result in inadequate stress transfer and distribution at the interface. This can lead to atrophy or bone resorption. This phenomenon is called "stress shielding".

Several ways have been suggested to overcome this problem. It can be assumed that the more closely the mechanical properties of an implant material resemble those of bone, a more controlled stress-strain field will be created by the implant in its bony environment. The choice of titanium for the manufacturing

of implants rests on this assumption [12, 47, 48].

The design of the implant can also effect the strain experienced by the bony tissues. Tangential or shear forces at the interface will impair the remodelling ability of the surrounding bone, but perpendicular forces will favour the bone response [11]. Therefore, the shape of an implant has to be chosen according to these principles. Examples of a biomechanically designed implant system are the Tübingen Frialit oral implant and the implant designed by Weiss [49].

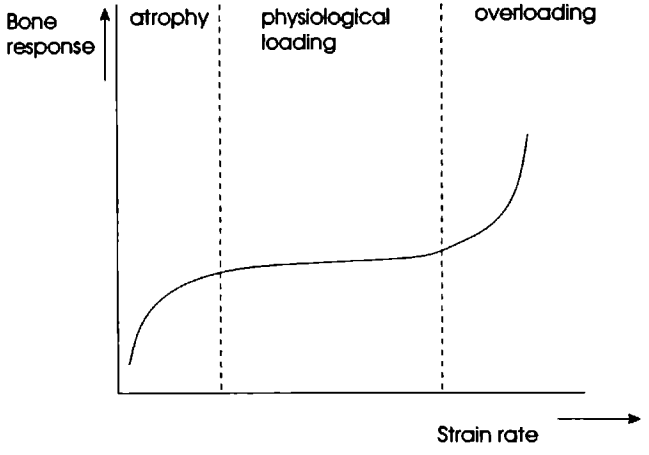


Figure 1.3: Schematic drawing of the Carter hypothesis. A hypertrophic response develops at high strain rates and bone atrophy occurs at low strain rates.

Another suggestion for obtaining more equal force distribution to the bone is the use of flexible elements in the implant [50, 51]. The reported advantages are:

1. simulation of the mobility of the periodontal ligament
2. damping of the applied forces

We must emphasise, however, that much of this is still speculative and unproved because of a lack of sufficient controlled clinical data.

1.3.4. Implant material

The focus in research on oral implants has been mainly on the biocompatibility of the materials used. Biocompatibility is defined as "the ability of a material to

perform with an appropriate host response in a specific application" [52]. For oral implants this means that the materials should at least be inert, capable of withstanding load, and resistant to corrosion.

Currently, commercially pure (c.p.) titanium and titanium alloy (Ti6Al4V) are the most commonly used metals in implant dentistry. C.p. Ti is composed of 99.75 % Ti, 0.05 % Fe, 0.10 % O, 0.03 % N, 0.05 % C, and 0.012 % H. Titanium is a very light weighted metal with a melting point of about 1665° C. Titanium also has a high corrosion resistance, thanks to the very inert and tenacious passivating film of titanium-oxide covering the metal [53]. This stable oxide layer is considered to be the most important factor for the favourable bone behaviour of titanium [54]. As described in section 1.3.3., an additional advantageous property is that the modules of elasticity of titanium is close to that of cortical bone. This might reduce non-physiological bone remodelling around the implants due to stress shielding. For a more extensive description of titanium as an implant material we refer to the textbook of Schroeder *et al.* [30] and the thesis of Johansson [55].

Other materials like ceramics have also been used. Ceramics are made by a combination of one or more metallic elements with one or more non-metallic elements. For implant purposes, ceramics can be classified into two varieties: bioinert and bioactive. Bioinert ceramics do not interact with the surrounding tissues and are non-soluble. An example of an inert ceramic for oral implants is aluminium oxide [11]. Bioactive ceramics are ceramics that, by their composition or solubility, participate in remodelling processes of the surrounding bony environment. Examples are calcium-phosphate (Ca-P) and glass ceramics.

The original interest in Ca-P ceramics for the construction of implant devices issued from their similarity to the mineral phase of bone tissue, i.e. hydroxyapatite (HA) and tricalcium-phosphate (TCP) [56, 57]. The most valuable biological advantage of Ca-P ceramics is their ability to become coated with a layer of bone mineral (carbonated apatite) after insertion into bone tissue [58, 59]. Normal remodelling of these bone layers occurs at the surface of the

ceramic deposited. The HA ceramic itself does not partake in the remodelling process nor is it resorbed by other processes.

In view of the favourable bone response, oral implants were manufactured of HA ceramics. In an initial series of experiments, the efficacy of HA ceramic root implants to maintain briefly the alveolar ridge after tooth extraction was proved [60]. Subsequently, artificial ceramic root implants were used for the fixation of prosthetic restorations and the retention of removable overdentures.

However, in the course of these studies it soon became clear that bulk HA ceramic had serious mechanical shortcomings [61]. HA ceramic has high resistance for compressive forces, but low tensile and bending strength. Therefore, implants of HA ceramic are not suitable in loaded situations.

To combine the excellent biological properties of HA with the strength of metal implants, it was proposed in the early eighties [62] to apply HA as a thin coating on a metallic surface. Currently, the plasma spray technique for deposition of Ca-P coatings is widely used for biomedical applications such as dental-root implants [63, 64]. The clinical use of HA-coated oral implants was supported by results from animal experiments. Various studies showed not only significantly higher percentages of bone contact along HA-coated implants compared with non-coated implants but also greater implant stability, which was confirmed by higher fixation strengths after short and prolonged implantation periods [65-74].

Despite these reported favourable results, there is still some concern regarding the viable use and prognosis of HA-coated implants. These concerns are:

- the substrate-to-coating fracture and fatigue strength properties and
- the observed degradation of the coating and the question of what will happen at the interface when the coating has disappeared.

The other bioactive ceramic material are glass ceramics. Their bone-bonding ability is based on the formation of a superficial gel-like, silicate-rich layer [75]. However, clinical studies have shown that these glass ceramics cannot be used for load-bearing implants since this gel-like layer cannot resist shear forces [11].

1.3.5. Implant surface conditions

Besides the mechanical and bulk chemical properties of the implant material, specific surface conditions can also affect the final bone response. Such important surface aspects are roughness and wettability.

As regards surface roughness, macroscopic and microscopic surface irregularities must be distinguished. Macroroughness, for example, as provided by screw threads and porous bead-shaped coatings, is mainly related to gross biomechanical stress and strain transfer between implant and bone [76, 77]. Microroughness (1-25 μm) can affect cell-implant interactions more directly. There is already considerable evidence from *in-vitro* experiments that cell shape and function are influenced by the substrate surface microtopography [78-81]. The effect on the *in vivo* bone response has also been studied. Several experiments have compared the performance of implants with as-machined and microroughened surfaces [82-86]. Although in some studies the implants did have additional mechanical benefit from screw threads, all rough surfaces showed a better bone response than did the as-machined surfaces.

The relationship between wettability and cellular behaviour has also been subject of many studies [87-90]. The general opinion is that hydrophilic surfaces improve cellular behaviour in terms of attachment, spreading, and growth. On the basis of the available knowledge, various chemical and physical methods, such as sulfonation and glow discharge, have been proposed to modify the surface energetic properties of an implant material to favour cellular response [91-93]. Moreover, there is evidence that improper implant handling, like inadequate sterilisation procedures or contamination during implant machining, can completely change the surface characteristics and result in an adverse tissue reaction [94, 95].

Recently, another surface treatment procedure has been suggested to improve the bone response to implants [96-98]. This approach is based on information obtained form more fundamental bone studies. Each step in bone formation is dependent on the presence of vitamins and essential nutrients. Further, hormones (e.g. thyroid and growth hormone) and specific proteins (e.g. bone

morphogenetic proteins) can influence bone-tissue development. Therefore, it may be assumed that coating of implant surfaces with growth stimulating proteins and hormones can enhance the bone-implant contact. Although the preliminary results with this new technique are promising, more research is required to confirm its clinical efficacy.

1.4. OBJECTIVE OF THE STUDY

As described above, several parameters influence the biological performance of oral implants. For example, faster bone bonding is obtained with HA-coated implants, but the relation between performance and design (e.g. crystalline *vs.* amorphous) of the coatings is not entirely clear [66, 99]. There is also some concern about the quality and long-term behaviour of coatings. For a definitive answer of these controversial topics, systematic and controlled studies have to be performed.

From the literature (see section 1.1) we know that the success rate for endosseous oral implants is lower in the maxilla than in the mandible. This poorer performance in the maxilla is mainly attributed to the less mineralised and less corticalised bone, so less primary stability at the implant-bone interface is achieved during the healing phase [4]. However, the increasing demand for implant-supported prosthesis encourages the search for new implant designs to improve the success rate for the maxilla especially for bone qualities types III and IV.

Consequently, the main experimental question adressed in this study is whether plasma-sprayed Ca-P coatings can improve the bone healing to oral implants inserted in low-density bone.

Three questions will be addressed:

- Do the original biomechanical bone characteristics influence the final bone behaviour? For this question, the bone behaviour to Ca-P-coated and non-coated implants inserted into the femoral and mandibular bone of goats will be examined.
- Does the application of Ca-P coatings improve the success rates of permucosal implants? To answer this question the tissue reaction to permucosal Ca-P-coated and non-coated implants inserted into low-density trabecular bone of the maxilla of goats will be examined.
- Does the application of Ca-P coatings reduce the required intervening healing time before loading of enossal oral implants? For this question the initial bone reaction to enossal-placed Ca-P-coated implants into the maxilla of goats will be investigated.

The experimental protocol used for these studies was approved by the Institutional Animal Care and Use Committee and adhered to the National Institutes of Health guidelines for the use of experimental animals.

REFERENCES

1. **R. Adell**, U. Lekholm, B. Rockler, and P.I. Brånemark, "A 15-year study of osseointegrated implants in the treatment of the edentulous jaw", Int. J. Oral Surg., 10, 387-416 (1981).
2. **R. Adell**, B. Ericksson, U. Lekholm, P.I. Brånemark, and T. Jemt, "A long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws", Int. J. Oral Maxillofac. Impl., 5, 347-359 (1990).
3. **T. Albrektsson**, G. Zarb, P. Worthington, and A.R. Eriksson, "The long-term efficacy of currently used dental implants: A review and proposed criteria of success", Int. J. Oral Maxillofac. Impl., 1, 11-25 (1986).
4. **T. Albrektsson** and L. Sennerby, "State of the art in oral implants", J. Clin. Periodontol., 18, 474-481 (1991).
5. **R.M. Meffert**, B. Langer, and M.E. Fritz, "Dental implants: A review", J. Periodontol., 63, 859-870 (1992).
6. **I. Naert**, "The influence of prosthetic design and implant type on tissue reactions around oral implants", Phd thesis, Leuven University Press (1991).
7. **L.C. Junqueira**, J. Carneiro and J.A. Long, "Functionele histologie", Wetenschappelijke Uitgeverij Bunge, Utrecht (1987).

8. **M.H. Ross** and L.J. Romrell, "Histology", Williams and Williams, Baltimore (1989).
9. **D.R. Carter** and D.M. Spengler, "Mechanical properties and composition of cortical bone", Clin. Orthop., 135, 192-217 (1978).
10. **K. de Groot**, "Bioceramics of calcium-phosphate", CRC Press, Boca Raton (1983).
11. **G. Heimke**, "Osseointegrated implants", CRC Press, Boca Raton, ed. G. Heimke, Fl., vol.1 (1990).
12. **L.V. Carlsson**, "On the development of a new concept for orthopaedic implant fixation", Phd thesis Göteborg (1989).
13. **R.K. Schenk**, "Cytodynamics and histodynamics of primary bone repair", In: Fracture healing (Dd. J.M. Lane), Churchill Livingstone, NY, 23-32 (1987).
14. **T. Albrektsson**, "Direct bone anchorage of dental implants", J. Prosthet. Dent., 50, 255-261 (1983).
15. **W.E. Roberts**, "Bone physiology and metabolism", In: C.E. Misch, Contemporary Implant Dentistry, Mosby, St.Louis, 327-354 (1993).
16. **T. Albrektsson** and L. Sennerby, "Direct bone anchorage of oral implants: clinical and experimental considerations of the concept of osseointegration", Int. J. Prosthodont., 3(1),30-41 (1990)
17. **T. Albrektsson**, P.I. Brånemark, H.A. Hansson, and J. Lindstrom, "Osseointegrated titanium implants", Acta Orthop. Scand., 52,155-170 (1981).
18. **M.S. Cune**, C. de Putter, J. Hoogstraten "Treatment outcome with implant retained overdentures: Part I: Clinical findings and predictability of clinical treatment outcome", J. Prosthet. Dent., 72(2),144-151 (1994)
19. **M.S. Cune**, C. de Putter, J. Hoogstraten "Treatment outcome with implant retained overdentures: Part II: Patient satisfaction and predictability of subjective treatment outcome", J. Prosthet. Dent., 72(2), 152-158 (1994).
20. **M. Quirynen**, I. Naert, D. van Steenberghe "Fixture design and overload influence marginal bone loss and fixture success in the Brånemark system", Clin. Oral Impl. Res., 3, 104-111 (1992).
21. **B. Engquist**, T. Bergendal, T. Kallus, and U. Linden, "A retrospective multicenter evaluation of osseointegrated implants supporting overdentures", Int. J. Oral Maxillofac. Impl., 3, 129-134 (1988).
22. **R.A. Jaffin** and C.L. Berman, "The excessive loss of Brånemark fixtures in type IV bone: a 5-year analysis", J. Periodontol., 62, 2-4 (1991).
23. **U. Lekholm** and G.A. Zarb, "Patient selection and preparation", In: P.I. Brånemark, G. Zarb, T. Albrektsson: Tissue integrated prosthesis: Osseointegration in clinical dentistry. Chicago; Quintessence Publ. Co. Inc. (1985).
24. **P. Thomsen**, L.M. Bjursten, S. Ahlstedt, U. Bagge, L.E. Erikson, "Proliferative synovitis in rabbit knee joints induced by antigen and preformed immune complexes", Scand. J. Rheumatol., 14, 239-251 (1985).
25. **W.E. Roberts**, K.E. Simmons, L.P. Garetto, R.A. De Castro, "Bone physiology and metabolism in dental implantology: risk factors for osteoporosis and other metabolic bone diseases.", Implant Dent., 1(1), 11-21 (1992).
26. **R.A. Eriksson**, R. Adell, "Temperatures during drilling for the placement of implants using the osseointegration technique.", J. Oral Maxillofac. Surg., 44, 4-7 (1986).

27 **P.I. Brånemark**, "Introduction to osseointegration", In "Tissue integrated prostheses, osseointegration in clinical dentistry", ed P I Brånemark, G A Zarb, T Albrektsson, Quintessence Publ Co Inc , 11-77 (1985)

28 **H. Ohashi**, M Therin, A Meunier, P Christell, "The effect of drilling parameters on bone (1) General healing response (225-231), (2) The influence of drilling site (232-236)", J Mater Science Mater in Med , 5, 225-231 (1994)

29 **J. Lundskog**, "Heat and bone tissue an experimental investigation of the thermal properties of bone tissue and threshold levels for thermal injury", Scand J Plast Reconstr Surg , 6 (suppl 9), 5-72 (1972)

30 **A. Schroeder**, F Sutter, D Buser and G Krebeler, "Orale Implantologie", Stuttgart (1994)

31 **W.E. Roberts**, "Bone tissue interface", J Dent Educ , 52, 804-809 (1988)

32 **R. Haider**, G Watzek, H Plenk, "Effects of drill cooling and bone structure on IMZ implant fixation", Int J Oral Maxillofac Impl , 8, 83-91 (1993)

33 **F. Sutter**, "Das ITI-Bonefit-Implantatsystem-Implantatbettgestaltung", In Chirurgische Zahnheilkunde, Die Quintessenz, 4, 541-554 (1991)

34 **R.L. Jacobs**, R D Ray, "The effect of heat on bone healing A disadvantage in the use of power tools", Arch Surg , 104(5), 687-691 (1972)

35 **L. Carlsson**, T Rostlund, B Albrektsson, T Albrektsson, "Implant fixation improved by close fit", Acta Orthop Scand , 59(3), 272-275 (1988)

36 **K. Søballe**, E S Hansen, H Brockstedt-Rasmussen, C M Pedersenn, C Bunger, "Hydroxyapatite coating enhances fixation of porous coated implants A comparison between press fit and non-interference fit", Acta Orthop Scand , 61(4), 299-306 (1990)

37 **P. Ducheyne**, L L Hench, A Kagan, M Martens, A Bursens, C Mulier, "Effect of hydroxyapatite impregnation on skeletal bonding of porous coated implants", J Biomed Mater Res , 14, 225-237 (1980)

38 **H. Oonishi**, M Yamamoto, E Tsuji, S Kushitani, M Aono, Y Ukon, "The effect of hydroxyapatite coating on bone growth into porous coated titanium alloy implants", J Bone Joint Surg (Br), 71, 213-216 (1989)

39 **J.A. Jansen**, J P C M van der Waerden, J G C Wolke, "Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbits", J Biomed Mater Res , 27, 603-610 (1993)

40 **H.M. Frost**, "Bone modelling and skeletal modelling errors", Springfield, Ill, Charles C Thomas Publishers (1973)

41 **M.S. Block** and J N Kent, "Endosseous implants for maxillofacial reconstruction", W B Saunders Company, Philadelphia (1995)

42 **D. Buser**, C Dahlin, R K Schenk, "Guided bone regeneration in implant dentistry", Quintessence Books, Chicago (1994)

43 **J. Wolff**, "Ueber die innere Architecturen des Knochens", Arch Path Anat , 50, 389-453 (1870)

44 **W. Roux**, "Beitrage zur Morphologie der funktionellen Anpassung", Arch Physiol Anat , 9, 120-158 (1885)

45 **O.T. Jensen**, "Guided bone graft augmentation", In Guided bone regeneration in implant dentistry ed D Buser, C Dahlin, R K Schenk, Quintessence Books, Chicago (1994)

46 **D.R. Carter**, W E Caler, D M Pingler, V H Frankel, "Fatigue behavior of adult cortical bone the influence of mean strain and strain range", Acta Orthoped Scand , 52, 481 (1981)

47 **J.B. Brunski**, "Biomaterials and biomechanics in dental implant design " Int J Oral Maxillofac Impl , 3, 85-97 (1988a)

48 **J.B. Brunski**, "Biomechanics of oral implants Future research directions ", J Dent Educ , 52, 775-787 (1988b)

49 **C.M. Weiss**, "A comparative analysis of fibro-osteal and osteal integration and other variables that affect long-term bone maintenance around dental implants ", J Oral Impl , 8(3) (1987)

50 **P.T. Tetsch**, K L Ackermann and A Kirsch, "Experience with intramobile cylinder implants a follow-up analysis ", Dental implants Materials and systems, ed G Heimke, 75-80 (1980)

51 **I. Van Rosen**, L H Braak, C de Putter, K de Groot, "Stress-absorbing elements in dental implants ", J Prosthet Dent , 64, 198-205 (1990)

52 **D.F. Williams**, "Titanium and titanium alloys ", In Biocompatibility of clinical implant materials, ed D F Williams vol 1, CRC Press Inc , Boca Raton, 9-44 (1987)

53 **B. Kasemo**, "Biocompatibility of titanium implants surface science aspects ", J Prosth Dent , 49, 832-837 (1983)

54 **B. Kasemo**, J Lausmaa, "Metal selection and surface characteristics ", In "Tissue integrated prostheses Osseointegration in clinical dentistry ", ed P I Brånemark, G A Zarb, T Albrektsson, Quintessence Publ Co , Inc , 99-116 (1985)

55 **C.B. Johansson**, "On tissue reactions to metal implants ", Phd thesis, Goteborg(1991)

56 **K. de Groot**, "Degradable ceramics ", In "Biocompatibility of clinical implant materials ", vol 1, CRC Press, Boca Raton, ed D F Williams, 199-224 (1981)

57 **H. Aoki**, "Science and medical applications of hydroxyapatite ", JAAS, Takayama Press, System Center co , Inc Tokio (1991)

58 **M. Jarcho**, J F Kay, K I Gumaer, R H Doremus, H P Drobeck, "Tissue, cellular and subcellular events at a bone ceramic hydroxyapatite interface ", J Bioeng , 1, 79-92 (1977)

59 **M. Jarcho**, "Calcium-phosphate ceramics as hard tissue prostheses ", Clin Orthop , 157, 259-266 (1981)

60 **H. Denissen**, "Dental root implants of apatite ceramics ", Phd thesis, Free University, Amsterdam (1979)

61 **C. de Putter**, "Permucosal dental implants of dense hydroxylapatite ", PhD thesis, Free University, Amsterdam (1984)

62 **K. de Groot**, R Geesink, C P A T Klein, P Serekian, "Plasma-sprayed coatings of hydroxylapatite ", J Biomed Mater Res , 21, 1375-1381 (1987)

63 **J.G.C. Wolke**, C P A T Klein K de Groot, "Bioceramics for maxillofacial applications ", In Bioceramics of the human body, eds A Ravaglioli, A Krajewski, Elsevier Applied Science, London, 166-180 (1992)

64 **J. Koeneman**, J Lemons, P Ducheyne, W Lacefield, F Magee, T Calahan, J Kay, "Workshop on characterization of calcium-phosphate materials ", J Appl Biomater , 1, 79-90 (1990)

65 **J.G.C. Wolke**, J M A de Blieck-Hogervorst, W J A Dhert, C P A T Klein, K de Groot, "Studies on the thermal spraying of apatite bioceramics ", J Thermal Spray Technol , 1, 75-82 (1992)

66 **J.F. Kay**, "Calcium-phosphate coatings for dental implants ", Dent Clin of North Am , 36, 1-18 (1992)

67 **J.A. Jansen**, H Caulier, J G C Wolke, J P C M van der Waerden, W Kalk, I Naert, "The effect of Ca-P coatings on trabecular bone response a histological and clinical study ", Proceedings 20th Annual Meeting of the Society for Biomaterials, Boston, 124 (1994)

68 **C.P.A.T. Klein**, P Patka, H B M van der Lubbe, J G C Wolke, K de Groot, "Plasma-sprayed coatings of tetracalcium-phosphate, hydroxylapatite and a-TCP on titanium alloy an interface study ", J Biomed Mater Res , 25, 53-65 (1991)

69 **C.P.A.T. Klein**, P Patka, J G C Wolke, J M A de Blieck-Hogervorst, K de Groot, "Long-term *in vivo* study of plasma-sprayed coatings on titanium alloys of tetracalcium-phosphate, hydroxyapatite, and a-tricalcium-phosphate ", Biomaterials, 15, 146-150 (1994)

70 **R.G.T. Geesink**, K de Groot, C P A T Klein, "Chemical implant fixation using hydroxyapatite coatings ", Clin Orthop , 225, 147-170 (1987)

71 **K. Søballe**, "Hydroxyapatite ceramic coating for bone implant fixation ", Acta Orthop Scand , Suppl 255 (1993)

72 **W.J.A. Dhert**, C P A T Klein, J A Jansen, E A van der Velde, R C Vriesde, P M Rozing, K de Groot, "A histological and histomorphometrical investigation of fluorapatite, magnesium whitlockite and hydroxyapatite plasma-sprayed coatings in goats ", J Biomed Mater Res , 27, 127-138 (1993)

73 **M. Gottlander**, T Albrektsson, L V Carlsson, "A histomorphometric study of unthreaded hydroxyapatite-coated and titanium-coated implants in rabbit bone ", Int J Oral Maxillofac Impl , 7, 485-490 (1992)

74 **S.D. Cook**, K A Thomas, J F Kay, M Jarcho, "Hydroxyapatite-coated titanium for orthopaedic implant applications ", Clin Orthop , 232, 225-243 (1988)

75 **L.L. Hench**, H A Passchal, "Direct chemical bone of bioactive glass-ceramic materials in bone and muscle ", J Biomed Mater Res Symp , 4, 25-42 (1973)

76 **J.E. Lemons** and R W Phillips, "Biomaterials for dental implants ", In C E Misch, Contemporary implant dentistry, Mosby, St Louis, 259-278 (1993)

77 **R.M. Pilliar**, D A Deporter, P A Watson, N Valiquette, "Dental implant design - effect on bone remodeling ", J Biomed Mater Res , 25, 467-483 (1991)

78 **R.M. Brunette**, "The effects of implant surface topography on the behavior of cells ", Int J of Oral and Maxillofac Impl , 3, 231-246 (1988)

79 **R. Shingvi**, G Stephanopoulos, D I C Wang, "Effects of substratum morphology on cell physiology - Review ", Biotechnology and Bioengineering, 49, 764-771 (1994)

80 **K.D. Chesmel**, J Block, "Cellular responses to chemical and morphologic aspects of biomaterial surfaces 1 ", J Biomed Mater Res , 29, 1089-1099 (1995)

81 **K.D. Chesmel**, C C Clark, C T Brighton, J Black, "Cellular responses to chemical and morphologic aspects of biomaterial surfaces 2 ", J Biomed Mater Res , 29, 1101-1110 (1995)

82 **M.S. Block**, J N Kent, J F Kay, "Evaluation of hydroxylapatite-coated titanium dental implants in dogs ", J Oral Maxillofac Surg , 45, 601-607 (1987)

83 **A. Wennerberg**, T Albrektsson, B Anderson, "An animal study of c p titanium screws with different surface topographies ", J Mater Science Mater In Med , 6, 302-309, (1995)

84 **A. Wennerberg**, T Albrektsson, B Anderson, J J Krol, "A histomorphometric and removal torque study of screw-shaped titanium implants with three different surface topographies ", Clin Oral Impl Res , 6(1), 24-30 (1995)

85 **D. Buser**, R K Schenk, S Steinemann, J P Fiorellini, C H Fox, H Stich, "Influence of surface characteristics on bone integration of titanium implants A histomorphometric study in miniature pigs ", J Biomed Mater Res , 25, 889-902 (1991)

86 **K. Gotfredsen**, A Wennerberg, C Johanson, L T Shougoard, E Hjorbig-Hanzen, "Anchorage of TiO_2-blasted, HA-coated, and machined implants An experimental study with rabbits ", J Biomed Mater Res , 29, 1223-1231 (1995)

87 **R.E. Baier**, A E Meyer, J R Natiella, R R Natiella, J M Carter, "Surface properties determine bioadhesive outcomes Methods and results ", J Biomed Mater Res , 18, 337-355 (1984)

88 **J.M. Schakenraad**, H J Busscher, C R H Wildevuur, J Arends, "The influence of substratum free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins ", J Biomed Mater Res , 20, 773-784 (1986)

89 **J.A. Jansen**, "Epithelial cell adhesion to dental implant materials", PhD thesis, Catholic University, Nijmegen (1984)

90 **J.A. Jansen**, J P C M van der Waerden, and K de Groot, "Fibroblast and epithelial cell interactions with surface-treated implant materials ", Biomaterials, 12, 25-30 (1991)

91 **M.M. Meenaghan**, J R Natiella, J L Moresi, H E Flynn, J E Wirth, R E Baier, "Tissue response to surface-treated tantalum implants Preliminary observations in primates ", J Biomed Mater Res , 13, 631-643 (1979)

92 **A.S.G. Curtis**, J V Forrester, C McInnes, F Lawrie, "Adhesion of cells to polystyrene surfaces ", J Cell Biol , 97,1500-1506 (1983)

93 **W.S. Ramsey**, W Hertl, E D Nowlan, N J Binkowski, "Surface treatments and cell attachement ", *In vitro*, 20, 802-808 (1984)

94 **A.E. Meyer**, R E Baier, P O J Glantz, J R Natiella, "Biomaterials selection, evaluation, and preparation ", In Dental Implants Principles and practice ed C A Babbush, W B Saunders Comp , Philadelphia, 31-42 (1991)

95 **J.H. Doundoulakis**, "Surface analysis of titanium after sterilization Role in implant-tissue interface and bioadhesion ", J Prosthet Dent , 58, 471-478 (1987)

96 **S. Downes**, C J Clifford, C Scotchford, C P A T Klein, "Comparison of the release of growth hormone from hydroxyapatite, heat-treated hydroxyapatite, and fluorapatite coatings on titanium ", J Biomed Mater Res , 29, 1053-1060 (1995)

97 **I. Onoi**, T Ohura, M Murata, H Yamaguchi, Y Ohnuma, and Y Kuboki, "A study in bone induction in hydroxyapatite combined with bone morphogenetic protein", Plast Reconstr Surg , 90, 870-879 (1992)

98 **T.H. Howell**, D Buser, J Fiorellini, H Stich, J Wozney, "Effect of bone morphogenetic protein on bone healing around endosseous implants ", J Dent Res , 74 (IADR Abstracts), abstract 115 (1995)

99 **C.A. van Blitterswijk**, Y P Bovell, J S Flach, H Leenders, I van den Brink, J de Bruijn, "Variations in hydroxylapatite crystallinity effects on interface reactions ", In Hydroxylapatite coatings in orthopaedic surgery, eds R G T Geesink, M T Manley, Raven Press, Ltd , NY, 33-47 (1993)

Chapter 2

THE EVALUATION OF CA-P COATINGS IN THE ANIMAL EXPERIMENT: THE IMPORTANCE OF THE STUDY DESIGN

J.A. Jansen[1], H. Caulier[1,2], M.A. van't Hof[3], I. Naert[2]

[1] Department Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[2] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[3] Department of Medical Statistics, Faculty of Medical Sciences, University of Nijmegen, The Netherlands

2.1. INTRODUCTION

A lot of research activities have been undertaken to improve the success rates of endosseous implants in the jawbone. To comply with limited and poor bone quality there can be a need for the use of modified implant designs and implants coated with bioactive ceramics. Interest in calcium-phosphate (Ca-P) ceramics for endosseous implants is derived from their relative similarity to the mineral phase of bone tissue, i.e. hydroxyapatite (HA) ($Ca_{10}(PO_4)_6(OH)_2$), octacalcium-phosphate (OCP) ($Ca_4H(PO_4)_32.5H_20$) and tricalcium-phosphate (TCP) ($Ca_3(PO_4)_2$) [1, 2]. The results of these experiments demonstrated that the most valuable characteristic of Ca-P ceramics is their ability to become coated with a microscopic layer of bone mineral after insertion into bone tissue [3, 4]. Various studies showed a faster and more intense bone adaptation to such coated implants [5-9]. Nevertheless, the reported results are not always consistent. Occasionally, they are even contradictory. Frequently, this is caused by an incorrect study design and the use of only qualitative evaluation criteria. For example, in a recent study, Steflik [10] evaluated various HA-coated and non-coated oral implants placed in the mandible of dogs. However, one of the implant types was inserted using a so-called one-stage procedure, while the other implants were placed using a two-stage procedure. These different procedures make the final behaviour of the implants less comparable. In addition, all experimental implants of each type and implantation period were positioned in the left and right mandible of just one dog. It cannot be ruled out that, by choosing this implantation scheme, observed differences in bone behaviour to the implants are just interanimal differences in bone healing.

Given this observation, the purpose of this chapter is to discuss methods and criteria for the objective standardised evaluation of Ca-P-coated materials for implantation in bone tissue.

Testing of the biological properties of Ca-P materials is performed by inserting the materials into the bone tissues of experimental animals [11]. Subsequently,

at the end of the experimental period and after retrieval of the specimens, several techniques are available to evaluate the tissue reaction, e.g., light-, transmission electron-, and scanning electron microscopy. Light microscopy allows to obtain information about the whole tissue part containing the implant, while transmission electron microscopy will only give information of a small area. Finally, scanning electron microscopy can be used for the evaluation of the material surface. A scanning electron microscope equipped with an EDS (energy dispersive spectroscopy) will provide chemical information about the implant/tissue interface. Considering these constrictions, light microscopy appears still the best method to assess the bone response to the implanted material.

In addition to this, for an accurate judgement of the biological behaviour of materials, it is necessary that the evaluation is performed in such a way that:

- the animal experiments are designed correctly,
- statistical analysis is comparative
- comparison of the result with other investigations is possible.

The first criterion can be fulfilled by designing the experiments in a proper methodological way by using appropriate techniques. Recent improvements in the histological sectioning techniques have made it possible that the second criterion can also be realised [12-14]. Finally, the third criterion can be cleared by using standardised histomorphometrical methods for the examination and assessment of the bone tissue-implant interface of the implant materials.

2.2. EXPERIMENTAL GROUP SIZE

The overall objective of experimental animal assays is to determine the *in vivo* behaviour of materials that are inserted for prolonged contact time with bone tissue. In addition, these tests can provide information about the reaction between material and gingival tissue [15]. In the case of Ca-P-coated implants, the experiments involve the enossal and permucosal insertion of these

implants into experimental animals (rabbit, goat, dog) for a predetermined time period. To determine statistical significant differences in biological performance, adequate observation periods and appropriate numbers of implant samples must be used.

For the calculation of the required sample size in an experiment, many formulas are presented in the literature [16,17]. The choice for the best formula depends on both the chosen experimental design and the "measurement level" of the observations. Actually, for the calculation of the required sample size, it is necessary to already know in advance which statistical technique will be applied for the final analysis of the obtained data.

In the present thesis for the histological evaluation of Ca-P-coated implants, quantitative techniques are used. Therefore, in this thesis calculations of required sample sizes are based on classic formulas [18].

The experiments on implants are mostly done in a split-plot-design, in which several observations are dependent of each other through the same experimental animal. Such split-plot-designs are considered to be more powerful than ordinary independent parallel group designs since each animal is "its own control". The gain in power in split-plot-designs depends on the magnitude of the interanimal correlation's, which may vary considerably over different experiments. In order to be sure, inter-animal correlation's are assumed to be small. This implies that the power of split-plot-designs and parallel-group-designs is assumed to be comparable for the purpose of power calculations.

This assumption makes it possible to apply a t-test (to compare two implant systems) or one-way-ANOVA (to compare several systems) for the evaluation of the experimental data. After carrying out an ANOVA, a mutual comparison of the groups must be done (Multiple Comparison Test) to identify the differences between the implant systems. A well-known method in the multiple comparison situation is the Bonferroni correction, which describes that the significance level of each combination tested is the aimed significance level (α = 0.05) divided by the number of tests performed [19]. This means that for the

calculation of the required experimental animal group size, the so-called two sample formula may be applied with an appropriate level of significance (α). The basic formula is given by [16]:

$$N/group = 2((Z\alpha + Z\beta)(\sigma/\delta))2$$

where:

σ = Dispersion of the observations in any population of implant system. Since the t-test or ANOVA is applied, all materials are assumed to produce the same variance (homoscedasticity). If this cannot be assumed, the different implant systems require different sample-sizes (beyond the scope of this paper).

δ = Threshold of relevance (or interest); e.g. δ is the smallest difference in bone contact percentage with biological relevance that has to be detected.

$Z\alpha$ = Critical t-value of the test (i.e., z-value of standard normal distribution). There are two considerations for the choice of $Z\alpha$. First, the use of a one- or two-sided test. Usually, two-sided tests are applied leading to $Z\alpha = 1.96$. However, in the case of implant systems, the hypothesis can also be tested one-sided, i.e.: only an improvement counts! This leads to $Z\alpha = 1.64$. Second, the number of tests to be done (Bonferroni-correction). Two strategies may be relevant in this respect. The first is to compare all implant systems mutually. Two implants lead to one test ($Z\alpha = 1.96$), 3 implants lead to three tests ($Z\alpha = 2.40$) and 4 implants to six tests ($Z\alpha = 2.64$), etc.

The second strategy is to compare all new implant systems with the old system as a control. Two new implants (i.e., 3 groups in the experiment) lead to two tests ($Z\alpha = 2.24$), and 3 new implants again result in $Z\alpha = 2.40$.

$Z\beta$ = Z-value associated with the power (β) of the test. β is the probability of obtaining a significant test result when the true difference is δ. β is usually set to 80 %, resulting in $Z\beta = 1.28$.

The choice for σ and δ is not simple in practice. The value of σ has to be taken from the literature or from previous experiences in comparable experiments. If there is no experience at all, a pilot study can be helpful in estimating σ.

Table 2.1 presents the required sample sizes for implants inserted in two different bone qualities. This histological evaluation parameter is bone contact percentage. Note that the decision to compare three implant systems instead of two does not increase the total number of implants by simply 50 % (for the third group), but by 70 % to compensate for the Bonferroni correction. For example in good quality bone, if δ = 8 (see Table 2.1, design B vs. C), comparison of 2 groups requires 2 x 33 = 66 implants and comparison of 3 groups requires 3 x 37 = 111 implants. Thus, the number of implant systems to be evaluated in an experiment is an important choice.

2.3. SURGICAL PROCEDURE AND CHOICE OF IMPLANTATION SITES

After ultrasonic cleaning in 100 % ethanol and sterilisation in an autoclave, the implants are inserted under aseptic conditions. Before implantation, the animals are sedated. Depending on the experimental site two different surgical procedures can be followed:

1. For implantation in extraoral bone tissue (tibia, femur) the implantation site is shaved. Then, the animals are placed on their back, the operation site is disinfected with povidone-iodine and the animals are completely surgically draped.
2. For intraoral implants extra precautions have to be made such as to avoid the contamination of the wound bed with saliva. The use of two suctions, one to suck the saliva the other to be used in the wound bed only, is highly recommended. The animals are completely sterile draped and the intraoral operation site is disinfected with a 0.1 % chlorhexidine solution.

For installation of the implants, longitudinal continuous incisions are made away from the implantation site to guarantee primary hermetical closure over the wound bed later.

The implant holes are carefully drilled with profuse saline cooling and low rotary speed to reduce the heating of the bone. Extra attention is paid to match the burr diameter with the implant size. Oversizing the hole results in an unstable implant. Subsequently, the implants are inserted. Finally, the wounds are carefully closed. The surgery has to be done always by the same operator. If required, animals were given antibiotics.

In addition to surgical technique, it has to be emphasised that the final biological reaction can be influenced by many external factors. These influences can be of biological or experimental origin, such as:

- health or general condition of the animals
- social behaviour of the animals (biting, grating, mating)
- local properties of the implant sites (trabecular bone, cortical bone, bone marrow)
- surgical fluctuations (presence of micro-organisms, size of incision)
- operator (experience, visibility)

Nevertheless, independent of their origin, all these influences contribute to the variability in the obtained data. The larger this variability is (σ), the larger the required sample size (see Experimental Group Size). Therefore, to balance such influences, sophisticated statistical methods have to be used for the allocation of the implants to assure a reliable randomisation of the implants.

Controlling for external influences can be obtained in two different ways:

1. Standardisation or exclusion: For example, by using only one gender, one age, one operator, one operation site, etc. The advantage of such a procedure is a low variance and small sample sizes, resulting in a good reproducibility and a high internal validity. A disadvantage is that the results of the experiments are hard to extrapolate (to other operators, gender, etc.). Consequently, the results only hold for one specific situation (low external validity).

Table 2.1 Required sample sizes per implant group for different thresholds of interest (δ) in different designs (A-G) when σ = 10 in good quality bone and σ = 40 in poor quality bone. The power is set to 80 % (of the designs A-G related to the normal two-tails probability (P-2-tails) associated Zα = 1.28), δ and σ in bone contact percentages. Interpretations with the aimed overall α = 0.05 and corresponding groups of implant systems in the experiment. Note that the total required number of implants is the sample size (from the table) multiplied by the number of groups. The required number of animals depend on the design (split-factor).

A P-2 tails = 0.10 - mutual comparison of 2 implants, 1-sided, 2 grps

B P-2 tails = 0.05 - mutual comparison of 2 implants, 2-sided, 2 grps - comparison of 2 implants with control, 1-sided, 3 grps

C P-2 tails = 0.033 - mutual comparison of 3 implants, 1-sided, 3 grps - comparison of 3 implants with control, 2-sided, 4 grps

D P-2 tails = 0.025 - comparison of 4 implants with control, 1-sided, 5 grps - comparison of 2 implants with control, 2-sided, 3 grps

E P-2 tails = 0.017 - comparison of 5 implants with control, 1-sided, 6 grps - comparison of 3 implants with control, 2-sided, 4 grps

F P-2 tails = 0.010 - comparison of 5 implants with control, 1-sided, 6 grps

G P-2 tails = 0.0083 - mutual comparison of 4 implants, 2-sided, 4 grps

	σ	δ	Zα	A 1.64	B 1.96	C 2.13	D 2.24	E 2.33	F 2.57	G 2.64
Good Bone Density	10	4		107	132	146	155	163	186	193
	10	5		69	84	94	100	105	119	123
	10	8		27	33	37	39	41	47	49
	10	10		18	21	24	25	27	30	31
	10	15		8	10	11	12	12	14	14
	10	20		5	6	6	7	7	8	8
Poor Bone Density	40	5		1092	1344	1489	1586	1669	1898	1967
	40	10		273	336	373	397	418	475	492
	40	15		122	150	166	177	186	211	219
	40	20		69	84	94	100	105	119	123
	40	25		44	54	60	64	67	76	79
	40	30		31	38	42	45	47	53	55
	40	35		23	28	31	33	35	39	41
	40	40		18	21	24	25	27	30	31

2 Balancing or stratification. Known influencing factors are equally allocated to the investigated implant systems. The factors included in the balancing procedure are unlikely to act as confounders, but a reduction in residual variance (σ^2) may still be obtained (at the expense of degrees of freedom in the statistical evaluation). The results of the

experiment can be generalised widely, thus yielding a high external validity.

For the evaluation of implant systems, it is most logical to apply the so-called "split-plot-design", i.e., the animals (= "plots") are "split" into several parts (operation sites), which are allocated to the implant systems in balance. The most important advantage of this split-plot design is that each animal can be used several times, which reduces the final number of experimental animals. In addition, factors such as gender, health and age are equally distributed over the implant systems so that confounding with respect to animal properties is excluded (each animal is its "own control").

Nevertheless, experimental influences (implantation site, operator, etc.) have to be taken into consideration. Balancing of these factors is best organised in a so-called "Latin square". This is only possible when the implantation sites are located at such a distance that mutual influences of the implants are excluded. This is also an argument for not splinting various types of oral implants once they are permucosally connected. Classic Latin squares are based on equal numbers of levels in the experimental factors.

Two examples of balanced, randomised implantation schemes are given in Tables 2.2 and 2.3 and in Figure 2.1.

In Table 2.2, four different implants are inserted into the right tibia of one experimental animal. The tibiae of the 16 animals are divided in four groups of four tibiae. In each group, for each tibia, the four implants are allocated to four positions (proximal to distal) using the Latin square design. This resulted in a randomisation of the factors animal number, position and implant type, while combinations of these factors cannot occur more than once.

Table 2.2 Four repeated Latin squares in the right tibia of 4 groups of 4 experimental animals The distribution of the implant materials (A, B, C, D) is homogeneous for both rows and columns

Group	I				II				III				IV			
Goat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Implant position																
Prox	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
↑	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A
↓	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B
Distal	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C

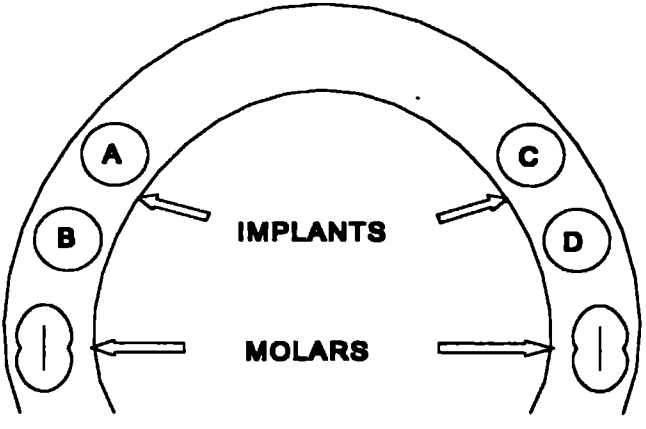


Figure 2.1 implant locations in the maxilla

Table 2.3: Two balanced split-plot designs in the maxillae of 6 experimental animals.

Left Frontal	Left Caudal	Right Frontal	Right Caudal
A	B	D	C
A	C	D	B
A	D	C	B
B	C	D	A
B	D	C	A
C	D	B	A

In Figure 2.1 and Table 2.3, four different implant materials are placed into left and right maxillary alveolar ridge of 6 experimental animals. The implants were located using a balanced split-plot design. This design is based on the assumption that there is no difference between the left and right alveolar ridge. Only, the implant locations frontal and caudal differ. Depending on σ and δ this design can be repeated in additional groups.

2.4. HISTOLOGICAL PREPARATION METHOD

At the end of the implantation period, the experimental animals are sacrificed by injection of Nembutal®. The implants, with their surrounding tissues are excised immediately, fixed in 10 % buffered formalin and embedded in methylmethacrylate after dehydration by alcohol series. For the light microscopical sectioning of these implant-containing specimens, two recently developed techniques can be used, i.e.:

1. "Sawing-grinding" technique as developed by Donath [12]
2. Modified inner circular "sawing" technique as developed by van der Lubbe [13] and Klein [14]

The equipment necessary for the sawing-grinding method is already commercially available (Exakt-cutting-grinding-system, Exakt Apparatebau, Germany). It consists of a precision guided, diamond-coated band saw and an

automatic grinding machine. With the band saw, a first cut is made through the polymerised block. On the exposed tissue-implant surface, a microscope slide is glued. This slide is mounted again in the band saw using a vacuum slide holder. Subsequently, a plan parallel section is cut of a thickness between 50-200 µm. This section is thinned down to a thickness of 5 to 10 µm with the grinding machine. Finally, the section is stained. For the staining, all the usually employed staining procedures for plastic embedded tissues can be used.

For the modified inner circular sawing technique, a horizontal interlock saw microtome (FMTA, The Netherlands) is used with many adjustments, i.e., freedom of movement of the saw blade, balanced rotation mechanism, thickness of sectioning. To prepare sections, the polymerised tissue/implant block is fixed in the specimen holder and a first cut is made to expose the implant surface. After staining the exposed specimen surface with basic fuchsin, Giemsa or methylene blue, a glass cover slip is fixed on the sample surface with cyanoacrylate-based glue. After drying, the cover slip, with the attached tissue/implant, is sawed off the block using a 1/1 mixture of glycerine and water as cooling liquid and lubricant. The sections obtained have a thickness between 5-10 µm. Finally, a glass slide is glued against the section.

Using the above described methods, it is possible to make thin sections of implant material and surrounding tissue without falling apart or damaging the interface. At the moment, it is difficult to express an opinion regarding which method is preferred; both methods have their merits. For example, the sawing-grinding technique results in sections of an occasionally very high quality. On the other hand, the sawing technique is less elaborate (no grinding or polishing) and allows the preparation of more sections of implant samples with a small diameter. For reason of availability, in our studies, we mainly use the sawing technique.

After the sectioning procedure, the obtained sections can be investigated by light microscopy. For estimation of the tissue response to the implants, histological and histomorphometrical evaluation can be performed. This

histological evaluation consists of a thorough description of the observed tissue reaction. The histomorphometry consists of quantitative analysis of the characteristics of the tissue surrounding the implants.

2.5. HISTOLOGICAL ANALYSIS

A light microscope is used for the qualitative histological analysis. The light microscope has to be equipped with a video camera and connected to a computer provided with an image analysis software package in order to perform quantitative histomorphometrical analyses.

For the quantitative histological evaluation of intra- and extraoral Ca-P-coated bone implants one or more of the following parameters can be assessed:

- The length of the gingival epithelium from the top of the implant abutment to the coronal boundary of the connective tissue.
- The thickness of the connective tissue from the apical limitation of the gingival epithelium to the marginal border of the alveolar bone crest.
- The percentage of direct bone contact between bone and implant.
- The amount of bone surrounding the implants.
- The thickness of the Ca-P coating. Based on the discriminating differences in gray level, an image could be depicted in which the coating is isolated from the underlying metal. Another technique makes use of 10 horizontal scan lines drawn perpendicular to the titanium surface. More detail about quantitative techniques is found in chapters 6 and 7.

Besides histomorphometry, other quantitative methods can be used like: dual X-ray absorptiometry (bone mineral density), X-ray radiography (measurement of bone levels, subtraction techniques), bone-implant interfacial strength measurements (push-out, pull-out, torque, Periotest®).

2.6. STATISTICAL ANALYSIS OF EXPERIMENTS

The statistical analysis of the proposed design is most efficiently done using analysis of variance (ANOVA). The outcome of the experiment (= dependent variable) is the final score of the tissue reaction on the implant material. As has been mentioned earlier, this reaction will be influenced by different experimental (side) factors. Such factors are called "nuisance variables" and are used only in the ANOVA for technical reasons (reduction in residual variance), but are not important for the interpretation of differences in the implant systems.

Table 2.2 is chosen as an example of the application of ANOVA in a Latin square design, leading to a 3-way ANOVA with 3 entrances (= independent variables):

1. The implant material (A, B, C, D)
2. The vertical position (proximal, distal)
3. The experimental animal (as shown in Table 2.2, each animal gives 1 x 4 observations per implant material; if 16 observations per implant system are required, then 16 animals are necessary).

ANOVA is preferably performed under the following assumptions:

- The residuals are normally distributed; in the case of a positive skewed distribution, a square root-transformation may be helpful.
- The implant sites within the same animal are independent of each other (i.e., no "carry-over" effects).
- Addition of the main effects = absence of interactions of any order between: animal, horizontal or vertical position, operator and implant system. These assumptions are often fulfilled, but the absence of interaction between operator and implant system may easily be violated due to possible prejudices of the operators. This must be taken into account when designing the experiment and selecting the operators or

implant systems (e.g., blindness; the operators must have no knowledge of the implant material they are inserting).

When using a computer in the calculations, the statistical package must allow for a variety of assumptions about interactions. This is the case in well-known packages such as SAS, SPPSX and BMPD.

- The available data must be complete. Missing data may lead to non-resolvable ANOVA schemes. Therefore, in the experimental design or surgical procedure, precautions must be taken to prevent loss of data. For example, by the application of antibiotics, separate housing of the animals, internal sutures to avert gnawing to the implantation wound, etc.
- A large number of nuisance-variables gives a considerable loss of degrees of freedom (DF). Only nuisance variables, which indeed reduce the residual variance, are useful. Otherwise, there is no compensation for the loss of DF and the power of the experiment is decreased. An efficient choice of nuisance variables is only possible after experiences in this field of experiments.
- If it is foreseen that the DF of the residual variance ends up at a lower level of about 30 DF, then Table 2.1 is not valid and the required sample size per group has to be increased by at least one observation (beyond the scope of this thesis).

When not all above discussed requirements can be fulfilled, a simpler design has to be chosen (less nuisance variables to be obtained by standardisation) or non-parametric methods in case of expected outliers (e.g., the Friedman-test = m-rankings) has to be applied. However, it must be stressed that non-parametric statistics results in a loss of power, which has to be compensated by more implants per group.

An overall significant result by ANOVA for the implant system has to be

followed by a multicomparison technique (e.g., Scheffé, Tukey, Newman-Keuls, Bonferroni, etc.)

2.7. DISCUSSION

Histomorphometry to measure the changes in the implant-tissue response can be used for the analysis of implanted biomaterials. However, the procedures to quantify the tissue response are usually very time-consuming and require a conscious methodology. The measurements are normally performed by point counting and linear intercepts using eyepiece graticules and light microscopy [20]. An alternative approach is the use of automatic or semi-automatic computer assisted image analysis systems, which are more efficient and may help save time. Unfortunately, these systems are not suitable for the assessment of implant-tissue interactions since they require a very high uniformity of sectioning and staining of the samples. Otherwise, there is a significant chance of recording mistakes or misinterpretation by the machine. Despite considerable improvements, it has to be concluded that it is presently still impossible to prepare such consistent high quality sections.

On the other hand, it is also known that a thorough histological description cannot be used as the only criterion for implant biocompatibility. Therefore, in this chapter already several parameters for the histomorphometrical evaluation are mentioned. These parameters will be discussed more in detail in the next chapters.

In this context, it should also be noted that although histological methods are important for the assessment of the biocompatibility of an implant material, the final tissue response can be affected by the experimental design. For example, to exclude the influence of implantation site in material evaluation studies, it is necessary that a complete and reliable randomisation schedule is used for the allocation of the implants. Therefore, in this chapter the use and potential of statistical methods for the evaluation of enossal implants were discussed. On

basis of these considerations and previous experiments, in the present thesis the following numbers of animals were used:

Chapter 3 (femur): number of goats (A-group): 12°
number of implants: 48* (12 HA/12 HAHT/12FA/12Ti (controls))
evaluation period: 3 months enossal

Chapter 5 (mandible): number of goats (A-group): 12°
number of implants: 48 (12 HA/12 HAHT/12 FA/ 12 Ti (controls))
evaluation period: 3 months enossal

Chapter 4 (Dexa): number of goats (A-group): 12°
number of implants: 48* (12 HA/12 HAHT/12 FA/12 Ti (controls))
evaluation period: 3 months enossal

Chapter 6 and 7 (pergingival histology + pergingival clinical):
number of goats (B): 16
number of implants: 64 (16 HA/16 HAHT/16 FA/16 Ti (controls))
evaluation period: 10 months (6 months enossal/ 4 months permucosal)

Chapter 8 (enossal): number of goats (C): 12
number of implants: 48 (12 HA/ 12 HAHT/ 12 FA/ 12 Ti (controls))
evaluation time: 3 months (n=6) and 6 months (n=6) enossal

Total number of goats for the whole experiment: 40

Total number of implants used for the whole experiment: 208

* concerns the same implants, ° concerns the same goats,

HA = hydroxyapatite, HAHT = heat treated hydroxyapatite,

FA = fluorapatite, Ti = titanium

REFERENCES

1 **K. de Groot**, "Degradable ceramics", Biocompatibility of Clinical Implant Materials, vol 1, (ed D F Williams), CRC Press, Inc , Boca Raton, 199-222 (1981)

2 **H. Aoki**, "Science and Medical Applications of Hydroxyapatite", JAAS, Takayama Press System Center co , inc Tokio (1991)

3 **M. Jarcho**, J F Kay, K I Gumaer, R H Doremus, and H P Drobeck, "Tissue, cellular and subcellular events at a bone-ceramic hydroxyapatite interface", J Bioeng , 79-92 (1977)

4 **M. Jarcho**, "Calcium-phosphate ceramics as hard tissue prosthetics", Clin Orthop and Related Res , 157 259-278 (1981)

5 **J.G.C. Wolke**, J M A de Blieck-Hogervorst, W J A Dhert, C P A T Klein, and K de Groot, "Studies on the thermal spraying of apatite bioceramics", J of Thermal Spray Technol , 1 75-82 (1992)

6 **J.F. Kay**, "Calcium-phosphate coatings for dental implants", Dent Clin of North Am , 36 1-18 (1992)

7 **M. Weinlaender**, E B Kenney, V Lekovic, J Beumer, P K Moy, and S Lewis, "Histomorphometry of bone apposition around three types of endosseous dental implants", Int J Oral Maxillofac Impl , 7 491-496 (1992)

8 **J.A. Jansen**, J P C M van der Waerden , J G C Wolke, "Histologic investigation of the biologic behavior of different plasma-sprayed coatings in rabbits", J Biomed Mater Res , 27 606-610 (1993)

9 **K. Søballe**, "Hydroxyapatite ceramic coating for bone implant fixation" Acta Orthop Scand , suppl 25 (1993)

10 **D.E. Steflik**, W R Lacefield, A L Sisk, G R Parr, F T Lake, and J W Patterson, "Hydroxylapatite-coated dental implants descriptive histology and quantitative histomorphometry", J of Oral Impl , 20 201-213 (1994)

11 **H.G. Lord**, "Techniques of Biocompatibility Testing " Boca Raton CRC Press, pp 5-34 (1986)

12 **K. Donath**, and G Breuner, "A Method for the Study of Undecalcified Bones and Teeth with Attached Soft Tissues", J Oral Pathol , 11 318-326 (1982)

13 **H.B.M. van der Lubbe**, C P A T Klein, K de Groot, "A Simple Method for Preparing Thin Histological Sections of Undecalcified Plastic Embedded Bone with Implants", Stain Technol , 63 171-177 (1988)

14 **C.P.A.T. Klein**, Y M H F Sauren, W E Modderman, J P C M van der Waerden, "A new saw technique improves preparation of bone sections for light and electron microscopy", J of Applied Biomater , 5 369-373 (1994)

15 **K. Langeland**, "Concise Encyclopedia of Medical and Dental Materials", Oxford Pergamon Press, pp 59-69 (1990)

16 **S.J. Pocock**, "Clinical Trials", Chichester John Wiley and Sons (1983)

17 **J.J. Schlesselman**, "Case-Control Studies", New York Oxford University Press (1982)

18. **Y.M.M. Bishop**, S E Fienberg, P W Holland, "Discrete Multivariate Analysis", Cambridge MIT Press (1975)

19 **R.J. Simes** "An Improved Bonferroni Procedure for Multiple Test of Significance", Biometrica, 73 751-754 (1986)

20 **P.A. Revel**, "Pathology of Bone", Berlin Springer-Verlag, pp 87-111 (1986)

Chapter 3

EFFECT OF CALCIUM-PHOSPHATE (CA-P) COATINGS ON TRABECULAR BONE RESPONSE; A HISTOLOGICAL STUDY

H. Caulier[1, 2], J.P.C.M. van der Waerden[2], Y.C.G.J. Paquay[2], J.G.C. Wolke[2,3], W. Kalk[2], I. Naert[1], J.A. Jansen[2]

[1] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[2] Department of Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[3] Department of Biomaterials, University of Leiden, The Netherlands

3.1. INTRODUCTION

During the last decades, an enormous progress has been made in oral implant dentistry. Currently, the use of oral implants may be considered an acceptable treatment concept to support dental prostheses [1-5]. Although there are differences in success percentages among them, various studies have demonstrated that implant success is related directly to the quality and quantity of the available jaw bone [6]. The clinical significance of the status of the host bed is best demonstrated by the observed difference in survival rate of implants inserted into the mandible and those inserted into the maxilla. For example, a recent study performed in the Netherlands demonstrated that the three-year implant survival rate for implant-retained overdentures in the lower jaw was 96.7 % while the survival percentage in the upper jaw was 70.7 % [7]. These percentages are independent of type of cylindrical implant used. The presence of low bone quality is supposed to be the major reason for the poorer performance of maxillary implants [8-9]. Lekholm and Zarb [10] visualised this problem by classifying the jaw bone quality. According to their classification, the maxilla consists mainly of loose trabecular bone surrounded by a thin cortical shell, so called Type III and Type IV bone. In contrast, the mandible consists primarily of a core of dense trabecular bone surrounded by a thick layer of compact bone.

Despite the clinically recognised importance of the host bed for the final success of oral implants, most experimental studies have been performed in high quality cortical bone. On the basis of the observed bone adaptation in these studies, currently, titanium and titanium-aluminium-vanadium-alloy are considered to be the materials of choice for oral implants [11]. On the other hand, it is known that the use of implants coated with a layer of calcium-phosphate (Ca-P) ceramic can increase the bone apposition [12-17]. Therefore it may be inferred that the application of Ca-P coatings can be beneficial for maxillary oral implants. In the present paper, we report the first results of a series of experiments conducted to test this hypothesis.

3.2. MATERIALS AND METHODS

3.2.1. Implant materials and coating characteristics

Forty-eight cylindrical titanium-aluminium-vanadium (Ti6Al4V) implants with a length of 10 mm were grit-blasted with Al_2O_3 (Ra = 4-5 µm). They were cleaned ultrasonically in propanol and dried at 100° C. Subsequently, the implants were left without coating or a Ca-P coating, approximately 50-60 µm thick, was applied using a plasma-spray process. Three different coatings were produced:

1. Hydroxyapatite (HA)
2. Hydroxyapatite coating subjected to heat-treatment (650° C during 10 minutes) (HAHT)
3. Fluorapatite coating (FA)

Plasma spraying was performed at a current of 400 A , a voltage of 70 V, and with a working distance of 13 cm. The arc and carrier gas were nitrogen.

For the deposition of both HA-coatings, commercially available spray dried powder (CAM IMPLANTS B.V., The Netherlands) with a mean particle size distribution of 38 µm, was used. For the FA-coating, fluorapatite powder ($Ca_5(PO_4)_3F$) was prepared by mixing $4Ca(OH)_2$ and $3H_3PO_4$ with CaF_2. The powder had a mean particle-size distribution of 22 µm. The coatings were characterised by X-ray diffraction (XRD) (Figure 3.1) and Fourier Transform Infra Red Spectroscopy (FTIR) (Figure 3.2). The flat defined baseline and the narrow peaks with high intensities in the diffractogram of FA are indicative of more crystalline material (95 %) with little or no amorphous content as compared with the HAHT-coated (crystallinity of 65 %, ratio of crystallinity to crystallinity + amorphous phases) and the HA-coated (crystallinity of 60 %) implants. Analysis of the IR spectra for the plasma-sprayed HA coating reveals a broad phosphate stretching region, which is indicative for the formation of some amorphous calcium-phosphate phases in the coating. The dehydroxylation that the HA coating has undergone partially disappeared in the FTIR of the HAHT coating because of the heat treatment after spraying.

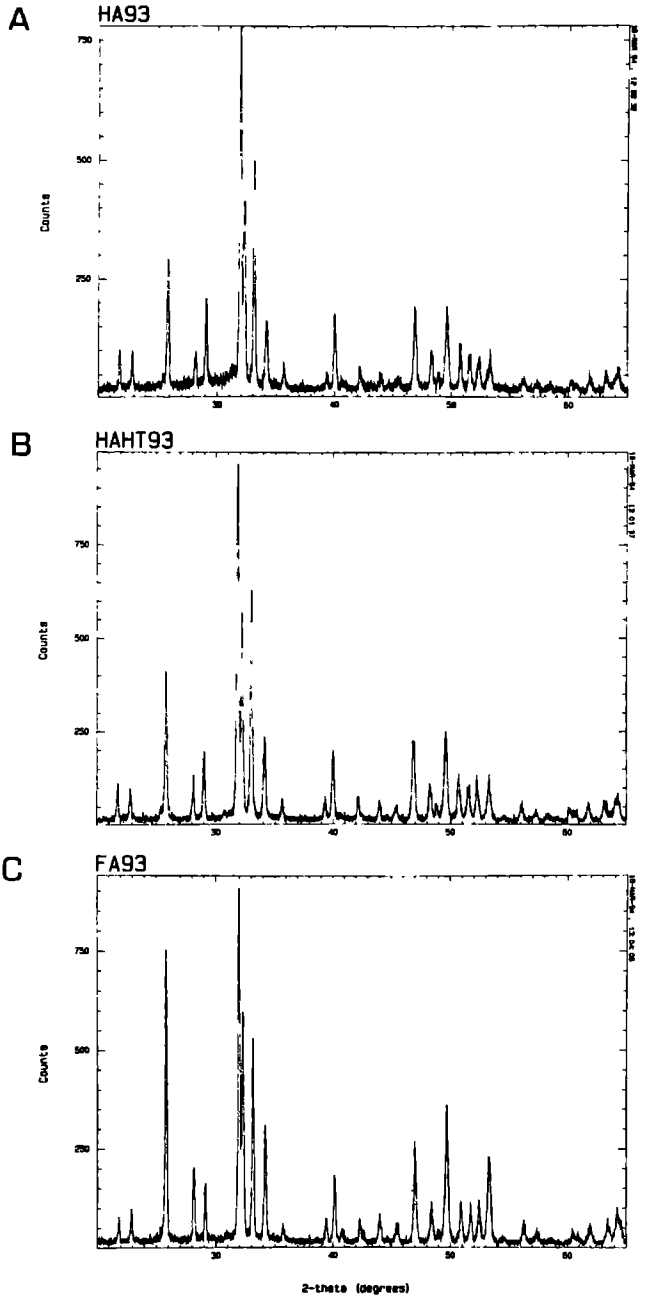


Figure 3.1: XRD patterns of HA (a), HAHT (b), and FA (c) plasma-sprayed coatings with the 2θ in degrees on the x-axis, and the total number of counts on the y-axis.

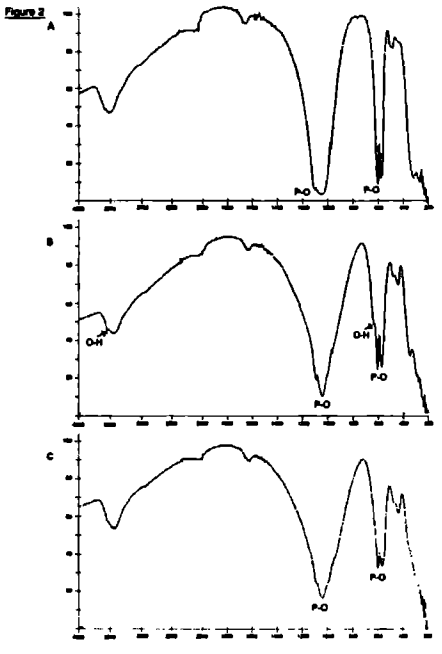


Figure 3.2: Infrared spectroscopy of plasma-sprayed HA (a), HAHT (b), and FA (c).

The diameter of the implants that were submitted for plasma-spray coating was 3.9 mm and the diameter of the implants that remained without coating (Ti) was 4.0 mm. Therefore, the final diameters of all implants were similar.

Before surgery, all implants were cleaned ultrasonically in 100 % ethanol to remove any loose particles and dried at 50° C. The implants then were sterilised in an autoclave.

3.2.2. Experimental design and surgical procedure

Twelve healthy adult female Saane goats, with an average age of 30 months and an average weight of 50-80 kg were selected for the experimental animal model. The animals were kept in quarantine for at least 4 weeks, and tested

for CAE/CL-arthritis.

The implants were placed into the trabecular bone of the femoral condyle. Surgery was performed under general anaesthesia, induced by intravenous penthobarbital (25 mg/kg/animal and atropine 0.5 mg/animal). After orotracheal intubation, anaesthesia was maintained by ethrane 2-3 % through a constant volume ventilator.

For the insertion of the implants, the animal was immobilised on its back and the hind limbs were shaved, washed and disinfected with povidone-iodine. A longitudinal incision was made on the medial and lateral surface of the left and right femur. After exposure of the femoral condyle, pilot holes were drilled in the trabecular bone. These holes were gradually widened with drills to the final diameter of the implants. The bone preparation was performed with a very gentle surgical technique and continuous internal cooling. Following the press-fit insertion of the implants, the soft tissues were closed in separate layers using resorbable Vicry® 2-0 sutures. A total of 48 implants was placed; 12 Ti, 12 coated HA, 12 coated HAHT, and 12 coated FA implants. Each animal received 4 implants, one in each lateral and medial side of the left and right femoral condyle. The implants were placed according to a balanced split plot design to compensate for differences in bone quality and load characteristics among implantation sites (see chapter 2).

To reduce the peroperative infection risk, prophylactic antibiotic Albipen® was administered for 3 days starting 1 hour postoperatively.

Six of the twelve goats received *in vivo* fluorochromes that adsorb to bone mineral during the time they are present in the blood circulation. These markers were administered at timed intervals (Table 3.1) to assess the mineralising surfaces of the bone.

3.2.3. Histological procedures

After twelve weeks, the animals were sacrificed using an overdose of Nembutal®. After killing the animals, the femoral condyles together with the implants were excised.

Table 3.1: Schedule indicating the time intervals at which six of the twelve goats received subcutaneous (s.c.) fluorochrome markers to indicate the remodelling activity after implant installation.

Weeks Before Sacrifice	Fluoro-chrome	Colour	Dose
7	Tetracycline	Yellow	22.5 mg/kg (s.c.)
5	Calceine	Green	20.0 mg/kg (s.c.)
1	Tetracycline	Yellow	22.5 mg/kg (s.c.)

Following fixation in 10 % buffered formalin solution, the specimens were dehydrated by alcohol series, and, finally embedded in methylmetacrylate. Non-decalcified thin (10 µm) sections were made using a modified diamond blade sawing microtome technique [18]. The sections were made perpendicular to the long axis of the implant. These sections were stained with methylene blue and basic fuchsine and examined by light microscopy.

Besides thin sections, 30 µm thick sections were prepared of the samples of the animals that received fluorochromes. These sections were rinsed with alcohol (96 % and 100 %), dried, and enclosed with Aquamount®. Finally, they were evaluated by fluorescense microscopy.

3.2.4. Histological evaluation

To evaluate the trabecular bone response to the implants, both histological and histomorphometrical measurements were performed. The histological evaluation consisted of thorough description of the observed tissue reaction. The histomorphometrical procedures were performed using a computer-based image analysis system (TCL-image). For the histomorphometrical evaluation, two parameters were assessed:

1. The percentage of bone contact.

For this purpose, the microscopic images were projected on a monitor using a video camera coupled to the light microscope (magnification 12.5 x). Subsequently, the amount of bone was measured for the total implant

perimeter. Finally, the percentage of bone contact was defined as the length of the interfacial area where there was direct bone-implant apposition. The histological sections for the quantitative bone evaluation were chosen randomly and were representative for the trabecular bone response. Three sections were used from each implant for bone contact analysis. Results presented are based on the average value of these three measurements.

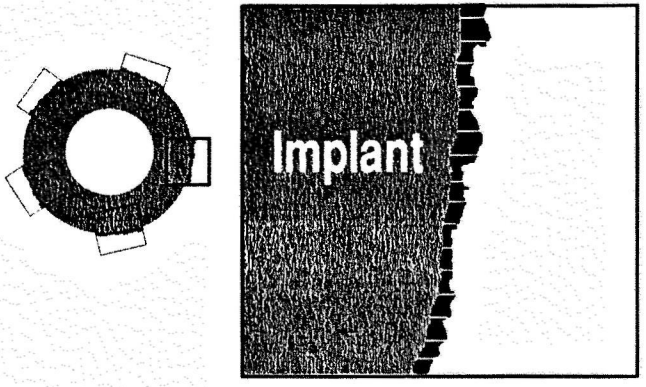


Figure 3.3: Schematic drawing of one of the five representative random images with discriminated coating on the implant surface, divided into 16 horizontal scan lines.

2. The thickness of the Ca-P coating.

For this measurement again the light microscope (magnification 8x), coupled with a video camera and computer, was used. Based on discriminating differences in gray level, an image could be depicted in which the coating was isolated from the underlying metal and surrounding tissues. The coating thickness was measured on three randomly selected sections of each implant type. Subsequently, five images in each section, covering about 80 % of the implant perimeter, were stored as digitised images in the computer. In these images the discrimination of the coatings by gray levels was fairly easy. Subsequently, 16 horizontal scan lines with a distance of 32 pixels were

superimposed on each image and the spacing between the coating boundaries was calculated (Figure 3.3) . This procedure resulted in a distance count of 80 for each implant. Finally, the data for the various coatings were merged and displayed in box- and whiskers plots.

3.3. RESULTS

One goat had to be sacrificed nine days after surgery due to a broken rib. The rest of the experimental animals had no complications and appeared to be in good health during the test period. At sacrifice, no clinical signs of inflammation or adverse tissue reactions could be seen around the implants. X-rays taken parallel to the long axis of the implant showed that the implants are located in trabecular bone. Only on the outside they are surrounded by cortical bone (see Figure 3.4).

3.3.1. Descriptive histological evaluation

Light microscopical evaluation.

The light microscopic evaluation of the implants demonstrated a satisfying bone maturation around the four different implants.

The non-coated Ti implants occasionally showed an intervening fibrous tissue layer at the bone-implant interface (Figure 3.5). At places were no fibrous encapsulation was present, close bone apposition to the implant surface was observed (Figure 3.6).

Around the FA-coated implants, an intimate bone-implant contact was formed. Sometimes, remodelling lacunae with osteoblasts were clearly visible (Figure 3.7). In none of these sections, did the FA coating show signs of reduction.

The bone reaction to the HA and HAHT (Figure 3.8) coated implants was similar to the FA-coated implants, frequently showing an intimate bone-implant contact. Also, remodelling lacunae with osteoblasts in contact with the HA and HAHT coating were observed.

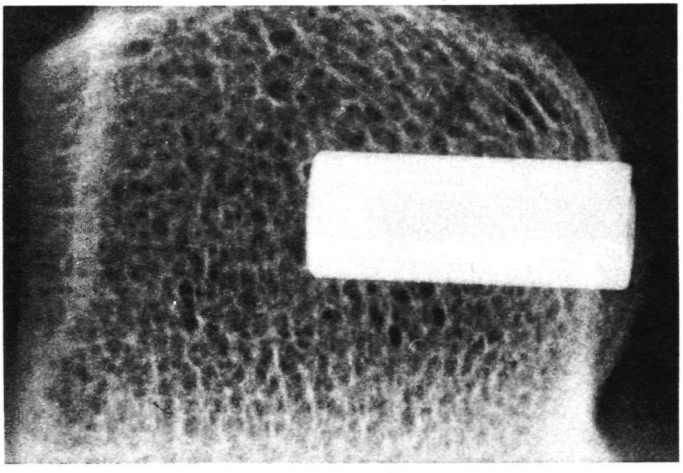


Figure 3.4: Radiograph of an implant located in the trabecular bone of the femoral condyle of the goat.

However, in contrast with the FA-coated implants, a moderate reduction in thickness of the HAHT coating (Figure 3.9) and a severe reduction of the HA coating were observed. This loss of HA and HAHT coating was not uniform and did not interfere with the intimate bone contact. Even on places where the coating was completely absent, a close contact of the bone with the underlying titanium alloy implant surface still existed without signs of fibrous tissue formation or inflammatory reaction (Figure 3.10).

Fluorescence microscopical evaluation.

The accumulation of tetracycline and calceine labelled bone demonstrated that an active remodelling activity had taken place in the vicinity of the implants. Newly formed bone was deposited on the coated as well as on the non-coated implant surfaces. In addition, no striking differences in bone remodelling activity around the various Ca-P-coated and non-coated implants were found (Figure 3.11).

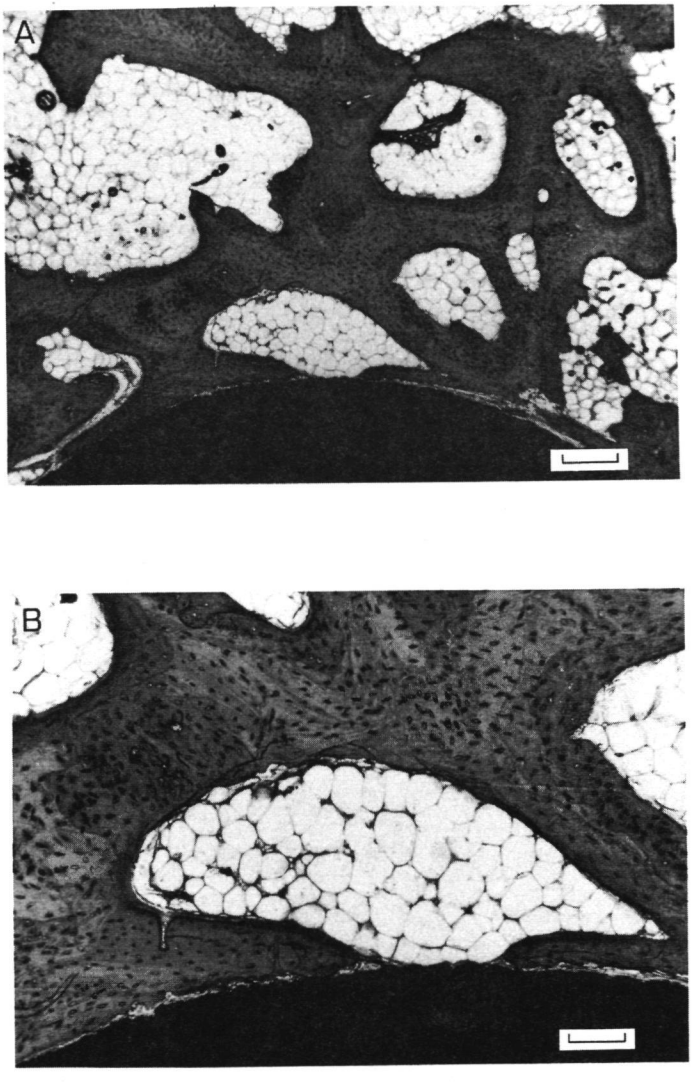


Figure 3.5: Histological appearance of a non-coated implant showing a fibrous tissue layer at the bone-implant interface. A: original magnification 10 x, bar = 294 µm; B: original magnification 25 x, bar = 118 µm.

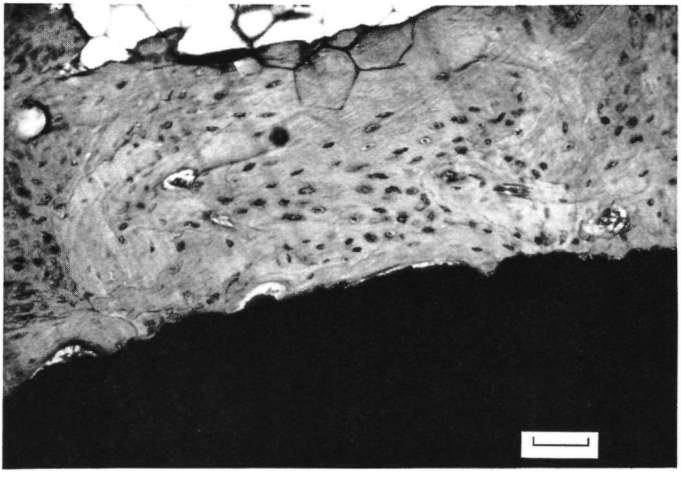


Figure 3.6: Light micrograph of an non-coated titanium implant showing an area of close bone-implant contact. No intervening fibrous tissue can be observed. Original magnification 40 x, bar = 73 µm.

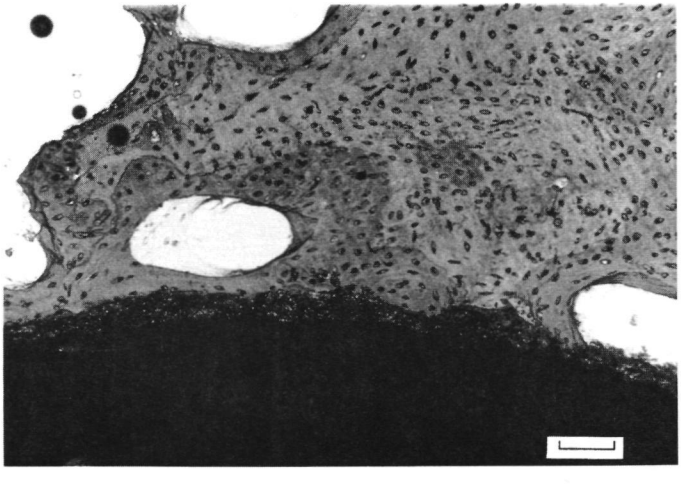


Figure 3.7: Light micrograph showing a fluorapatite-coated implant. An intimate bone contact at the interface can be observed. Note also the unchanged thickness of the FA-coating. Original magnification 32 x, bar = 32 µm.

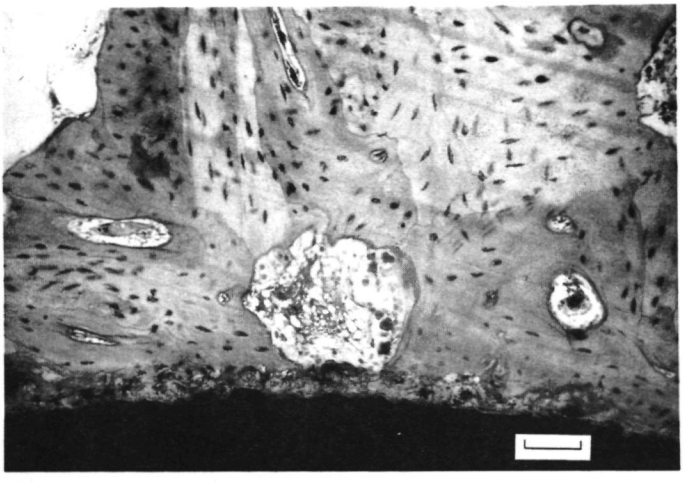


Figure 3.8: Histological appearance of a HAHT-coated implant demonstrating bone deposition on the implant surface and cellular activity in the remodeling lacuna on the implant surface. Original magnification 40 x, bar = 73 µm.

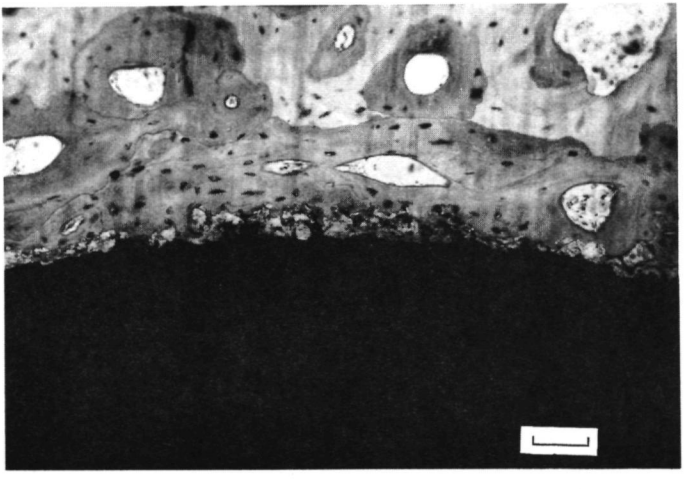


Figure 3.9: Histological section of a HAHT-coated implant. The reduction in coating thickness is clearly visible. Original magnification: 40 x, bar = 73 µm.

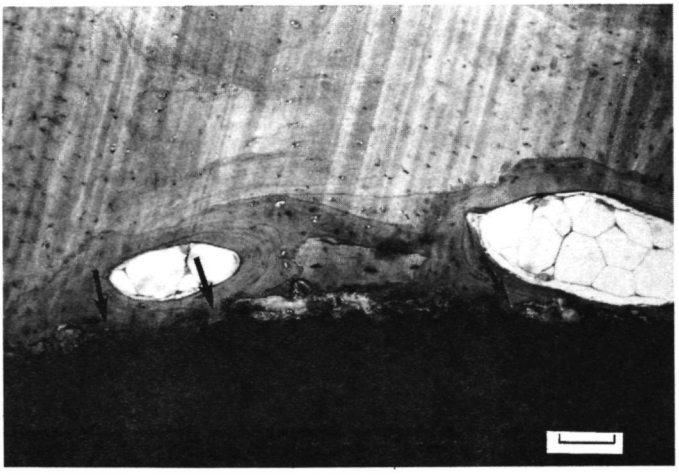


Figure 3.10: Histological section of a HA-coated implant. On some places the coating has completely disappeared (arrows). Nevertheless, there still exists a close bone contact with the original implant surface. Original magnification 40 x, bar = 73 µm.

3.3.2. Histomorphometrical evaluation

Percentage of bone contact.

The histomorphometrical analysis demonstrated a variation up to 20 % in the amount of bone contact between the three used sections of each implant. Table 3.2 and Table 3.3 show all bone apposition data for the various implants and implantation sites. Statistical testing, using an one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman-Keuls) revealed a significant difference in bone contact between implants inserted in medial and those inserted in lateral condyles ($p < 0.05$). Further, statistical testing indicated that also the difference in bone apposition between Ca-P-coated and non-coated implants was significant ($p < 0.001$). No significant difference in percentage of bone contact existed among the FA, HA and HAHT-coated implants.

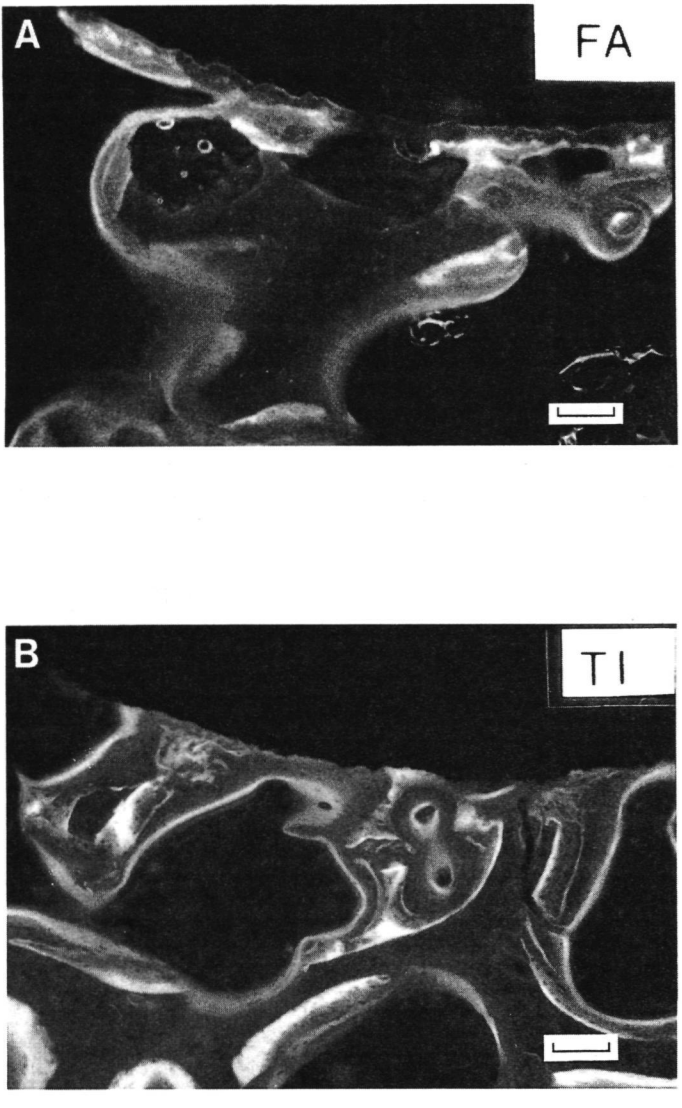


Figure 3.11: Fluorescence micrograph of a FA-coated (A) and a non-coated (B) implant. Lamellar bone is deposited on both implant surfaces. In addition, no difference in bone remodeling activity between the two types of implants could be observed. Original magnification 25 x, bar = 85µm.

Table 3.2: Mean Bone Apposition (%) ± Standard Deviation of the Four Different Implant Types.
The number of implants that were studied for each group is eleven.

Mean % of Bone Contact ± Standard Deviation	
FA	78.4 ± 9.1
HAHT	79.1 ± 8.15
HA	78.2 ± 9.4
TI	56.8 ± 16.9

Table 3.3: Means and Standard Deviations for the Various Implants for the Two Implantation Sites.
*The number of the measured histological sections is shown between brackets.

	FA	HAHT	HA	TI
MEDIAL	82.36 ± 7.2 (n=18)*	80.58 ± 10.7 (n=15)	84.34 ± 5.5 (n=15)	60.82 ± 12.7 (n=18)
LATERAL	72.8 ± 10 (n=15)	76.98 ± 8.5 (n=18)	74.03 ± 11.0 (n=18)	49.34 ± 21.4 (n=15)

Thickness of the Ca-P coating.

In Figure 3.12 the results from the measurements of coating thickness are shown in box- and whiskers plots, using a Tukey five number summary (0, 25, 50, 75, and 100th percentile). The box contains 50 % of the data and includes a line for the median. The whiskers represent the lower and upper 25 % of the data. The plots show that the data for all coatings are skewed. The plots also illustrate that all coatings show reduction in thickness. However, this reduction is not uniform. In some areas the coating thickness was not reduced and the original thickness maintained. In contrast, in other areas the coating was reduced considerably. Occasionally, the HA- and HAHT coatings even completely disappeared. Further, the median drawn in the middle of the boxes tells that overall the HA coating shows the most reduction in coating thickness.

3.4. DISCUSSION

Animal models used to study the cortical bone behaviour in relation to implant materials are not very effective for the investigation of the behaviour of maxillary oral implants. Consequently, in this study a trabecular experimental design was used to evaluate the possible beneficial bone effect of three calcium-phosphate coatings in comparison with non-coated Ti-alloy implants. Measurements of the percentage of bone contact demonstrated significantly more bone apposition to the Ca-P-coated than to the non-coated implants.

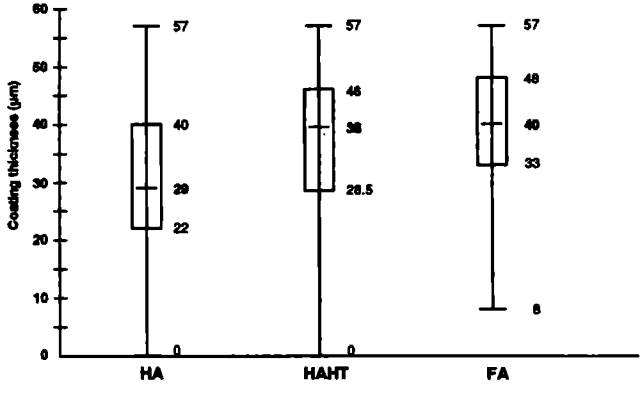


Figure 3.12: Box-whisker plots showing the results from the compute-based analysis of the coating-thickness reduction for the three different coatings.

Other studies have demonstrated similar observations with Ca-P-coated implants [19-21], so this finding is not surprising. However, this study was unique, in that we used a very careful surgical technique that resulted in minimal bone damage during drilling and in optimal fit of the implants. Therefore the only factor in our study that could affect the final bone response to the various implant materials was the status of the host bone at the two

implantation sites. The significance of this parameter clearly was shown by the observed difference in bone apposition to implants in lateral condyles as compared with implants in medial condyles. An explanation for this finding might be that the bone turn-over and the original bone mineral density of the lateral and medial condyles differ. This observation confirms once again the need of proper statistical implantation schedules in bone-biocompatibility tests (see chapter 2).

Other problems associated with the testing of materials for bone replacements are the histological preparation and evaluation techniques. The modified sawing technique used in this study appears to be an excellent method for the preparation of light microscopical sections of hard implant materials, including the surrounding tissues. A high number of high quality sections of each implant can be produced, and also the staining technique used provides good contrast for differentiating between the various tissues and implant materials.

Evaluation of three representative sections taken at different levels from the same implant revealed a significant variance in trabecular bone response. This observation refutes the statement of Søballe [22] who assumed that one section from each implant is sufficient for histomorphometrical analysis. This study indicates that completely different conclusions about the suitability of an implant material may be reached dependent upon the level evaluated.

With regard to the fluorescence microscopy, in this study, no additional information was obtained about the bone apposition and remodelling processes around the implants. This observation proves that fluorescent labels are not very useful when implants are placed in press-fit conditions.

Further, we made transversal histological sections of our cylindrical implants. This made it possible to evaluate the complete circumferential implant-tissue contact area. Unfortunately, due to the different preparation steps, the top or bottom of the implants, in relation to the main load direction is unknown. Nevertheless, it cannot be excluded that the observed 58 % bone contact to the bioinert non-coated titanium alloy implants is caused mainly by an unidirectional loading condition in the interface. Therefore, as also confirmed

by an earlier report of Heimke *et al* [23], a definitive statement about the behaviour of trabecular bone along the surface of titanium alloy implants can only be made with the help of experiments in absolutely load free conditions (see chapter 5). From the standpoint of bone response, this last phenomenon demonstrates the importance of further development of coating techniques for the deposition of bioactive Ca-P ceramics on bioinert implant materials.

An interesting finding in the present study resulted from the measurement of the residual coating thickness. Although several reports already have been published about the stability of plasma-sprayed Ca-P coatings [24-25], in those studies mainly subjective parameters were used to quantify the coating behaviour. Only occasionally were histomorphometric techniques applied [19, 26]. A drawback inherent in the quantification methods used in other studies is the presentation of the results as an overall mean of the coating thickness. It was recognised in our study that the reduction in coating thickness was not uniform, and by using the proposed analysis, this lack of uniformity was clarified. Similar to other studies, the results confirmed the relative stability of FA coatings; in contrast to other studies, however, the measurements showed a great variance in thickness reduction within each material group. For example, for the HA-plasma-sprayed implants, on some locations the coating had completely disappeared, while in other areas the coating was still intact. The final clinical consequences for this variable coating behaviour are not clear, since we observed that the bone-implant contact was not influenced by this coating loss. Recently Kangasniemi *et al* [27] reported that plasma-sprayed Ca-P coatings should not be used in long-lasting load-bearing application. They measured the bone bonding strength of FA and HA-coated titanium implants using a tensile test. Fractures always occurred in the coating-titanium interface. Consequently, irrespective of the desire to develop long-lasting coatings, it can be hypothesised that a thinner coating or a coating that shows a predictable homogenous disintegration is more favourable. This assumption is supported by the fact that Ca-P coatings show an effect only on the initial bone response [21].

3.5. CONCLUSION

In summary, we conclude that Ca-P coatings have no negative influence on bone-implant contact, not even when the coating disappears, and that coated implants enhance the quantity of direct bone formation on the implant surface in comparison with the non-coated implants. Based on the findings of this study, we suggest that implants combined with a Ca-P coating may decrease the implant failures in the maxilla. In future studies we hope to confirm this hypothesis.

REFERENCES

1. **R. Adell**, U. Lekholm, B. Rockler, and P.I. Brånemark, "A 15-year study of osseointegrated implants in the treatment of the edentulous jaw", Int. J. Oral Surg., 10, 387-416 (1981).
2. **R. Adell**, B. Eriksson, U. Lekholm, P.I. Brånemark, and T.Jemt, "A long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws", Int. J. Oral Maxillofac. Impl., 5, 347-359 (1990).
3. **T. Albrektsson**, G. Zarb, P. Worthington, and A.R. Eriksson, "The Long-Term Efficacy of Currently Used Dental Implants: A Review and Proposed Criteria of Success", Int. J. Oral Maxillofac. Impl., 1, 11-25 (1986).
4. **T. Albrektsson** and L. Sennerby, "State of the Art in Oral Implants", J. Clin. Periodontol., 18, 474-481 (1991).
5. **T. Albrektsson**, P.I. Brånemark, H.A. Hansson, and J. Lindström, "Osseointegrated titanium implants", Acta Orthop. Scand., 52, 155-170 (1981).
6. **P.A. Schnitman**, "Implant Dentistry: Where are we now?", JADA, 124, 39-47 (1993).
7. **M.S. Cune**, "Treatment outcome with implant-retained overdentures: part I and II", J. Prosthet. Dent., 72, 144-158 (1994).
8. **B. Engquist**, T. Bergendal, T. Kallus, and U. Linden, "A Retrospective Multicenter Evaluation of Osseointegrated Implants Supporting Overdentures", Int. J. Oral Maxillofac. Impl., 3, 129-134 (1988).
9. **R.A. Jaffin** and C.L. Berman, "The Excessive Loss of Brånemark Fixtures in Type IV Bone: A 5-Year Analysis", J. Periodontol., 62, 2-4 (1991).
10. **U. Lekholm** and G.A. Zarb, "Patient selection and Preparation", In: P.I. Brånemark, G. Zarb, T. Albrektsson: Tissue integrated prosthesis: Osseointegration in clinical dentistry. Chicago; Quintessence Publ. Co. Inc. (1985).
11. **R.M. Meffert**, B. Langer,and M E. Fritz, "Dental Implants: A Review", J. Periodontol., 63, 859-870 (1992).
12. **K. de Groot**, R.G.T. Geesink, C.P.A.T. Klein, and P. Serekian, "Plasma-

sprayed coatings of hydroxylapatite", J. Biomed. Mater. Res., 21, 1375-1381 (1987).
13. **R.G.T. Geesink**, K. de Groot, and C.P.A.T. Klein, "Chemical implant fixation using hydroxylapatite coatings", Clin. Orthop., 225, 147-170 (1987).
14. **K.A. Thomas**, J.F. Kay, S.D. Cook, and M. Jarcho, "The effect of surface macrotexture and hydroxylapatite coating on the mechanical strengths and histologic profiles of titanium implant materials", J. Biomed. Mater. Res., 21, 1395-1414 (1987).
15. **S.D. Cook**, K.A. Thomas, J.F. Kay, and M. Jarcho, "Hydroxyapatite-coated titanium for orthopaedic implant applications", Clin. Orthop., 232, 225-243 (1988).
16. **K.A. Thomas**, S.D. Cook, R.J. Haddad, J.F. Kay, and M. Jarcho, "Biologic response to hydroxylapatite-coated titanium hips. A preliminary study in dogs", J. Arthroplasty, 4(1), 43-53 (1989).
17. **W.J.A. Dhert**, C.P.A.T. Klein, J.G.C. Wolke, E.A. van der Velde, K. de Groot, and P.M. Rozing, "A mechanical investigation of fluorapatite, magnesiumwhitlockite and hydroxylapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 25, 1183-1200 (1991).
18. **H.B.M. Van Der Lubbe**, C.P.A.T. Klein, and K. de Groot, "A Simple method for preparing thin (10 µm) histological sections of undecalcified plastic embedded bone with implants", Stain Technol., 63, 171-177 (1988).
19. **W.J.A. Dhert**, C.P.A.T. Klein, J.A. Jansen, E.A. van der Velde, R.C. Vriesde, P.M. Rozing, and K. de Groot, "A histological and histomorphometrical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 27, 127-138 (1993).
20. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, and K. de Groot, "Histologic evaluation of the osseous adaptation to titanium and hydroxyapatite-coated titanium implants", J. Biomed. Mater. Res., 25, 973-989 (1991).
21. **K. Søballe**, E.S. Hansen, H. Brockstedt-Rasmussen, C.M. Pedersen, and C. Bünger, "Hydroxyapatite coating enhances fixation of porous coated implants. A comparison in dogs between press-fit and noninterference fit", Acta Orthoped. Scand., 4, 299-306 (1990).
22. **K. Søballe**, "Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs", Acta Orthopaed. Scand., Supplementum no. 255, 64 (1993).
23. **G. Heimke**, P. Griss, E. Werner, and G. Jentschema, "The effects of mechanical factors on biocompatibility tests", J. Biomed. Engin., 3, 209-214 (1981).
24. **C.P.A.T. Klein**, J.M.A. de Blieck-Hogervorst, J.G.C. Wolke, and K. de Groot, "-Studies of the solubility of different calciumphosphate ceramic particles *in vitro*", Biomaterials, 11, 509-512 (1990).
25. **M. Jarcho**, "Retrospective analysis of hydroxyapatite developement for oral implant applications", Dent. Clin. North. Am., 36(1), 19-26 (1992).
26. **D.B. Burr**, Satoshi Mori, R.D. Boyd, T.C. Sun, J.D. Blaha, L. Lane, and J. Parr, "Histomorphometric assessment of the mechanisms for rapid ingrowth of bone to HA/TCP coated implants", J. Biomed. Mater. Res., 27, 645-653 (1993).
27. **I.M.O. Kangasniemi**, C.C.P.M. Verheyen, E.A. van der Velde, and K. de Groot, "*In vivo* tensile testing of fluorapatite and hydroxylapatite plasma-sprayed coatings", J. Biomed. Mater. Res., 28, 563-572 (1994).

Chapter 4

THE ASSESSMENT OF BONE SURROUNDING IMPLANTS IN GOATS: *EX VIVO* MEASUREMENTS BY DUAL X-RAY ABSORPTIOMETRY

F G A Corten[1], H Caulier[1,2], J P C M van der Waerden[1], W Kalk[1], F H M Corstens[3], J A Jansen[1]

[1] Department of Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[2] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[3] Department of Nuclear Medicine, University of Nijmegen and University Hospital Nijmegen, The Netherlands

4.1. INTRODUCTION

Clinical survival percentages of implants have been clearly associated with the quality of the bony environment that surrounds the implants [1, 2]. In addition, it is known that the use of calcium-phosphate (Ca-P) ceramic for the manufacturing of implants can result in an improved bone response [3, see chapter 3]. The precise mechanism underlying this biological advantage of Ca-P ceramics is not yet completely understood.

In most of the studies on bone growth around oral implants, histology is used to assess the bone reaction. A less aggressive technique would be more appropriate, since it would make it possible to acquire information not only about the final quality of the newly formed bone but also during the course of the healing response.

In addition to the histological appearance, an important measure for bone quality is the bone mineral density (BMD) [4]. However, according to Keratli *et al* [5] BMD cannot be reliably determined by means of conventional techniques. For example, deviations in density of more than 30 % are observed with radiography. Another method that has been reported to be more reliable for evaluating the bone-mineral status of skeletal bone is Dual Energy X-ray Absorptiometry (DEXA). In several studies, the efficacy of this technique for the measurement of bone condition changes with porous implant materials [6] and at defined distant areas around femoral hip implants has already been demonstrated [5-8]. Although, this approach is very well suited for the overall monitoring of implant behaviour, quantification of the BMD within a distinct and narrow zone close to the implant should provide more predictable information about the actual bone response. Therefore, supported by recent software improvements for quantifying BMD, the aim of the present study is to determine the applicability of the DEXA technique for determining the influence of implant materials on the interfacial bone-healing capacity. *In vitro* BMDs were measured directly adjacent to and at various distances from various Ca-P-coated implants inserted into the trabecular bone of the femoral condyles of goats. In addition, information about the

influence of the Ca-P ceramics on the trabecular bone response was obtained. To determine whether BMD measurements are indeed predictive for the bone behaviour around implants, the results were compared with histological and histomorphometrical data.

4.2. MATERIALS AND METHODS

4.2.1. Implants

Twelve healthy, adult, female Saane goats with an average age of 30 months and a weight of 50 - 80 kg were used. The animals were kept in quarantine for at least 4 weeks prior to the experiment and tested for CAE/CL arthritis.

For the experiment, cylindrical Ti6Al4V (Ti) implants measuring 10 mm in length were manufactured. A central cylindrical opening was made on one side of the implants. All the implants were grit-blasted with Al_2O_3 (Ra = 4-5 µm). They were cleaned ultrasonically in propanol and dried at 100 °C. Subsequently, they were left either non-coated or coated with a Ca-P film, approximately 50-60 µm thick, by means of a plasma-spray process. Three different coatings were applied:

1. Hydroxyapatite coating with a crystallinity of 60 % (HA).
2. Hydroxyapatite coating subjected to heat treatment (650 °C for 10 min) resulting in a slight increase of the crystallinity to 65 % (HAHT).
3. Fluorapatite coating with a crystallinity of 95 % (FA).

The final diameter of the coated and uncoated implants was 4 mm. The coatings were characterised by X-ray diffraction (XDR) and infra-red spectroscopy (IR).

Prior to surgery, all implants were cleaned ultrasonically in 100 % ethanol to remove any loose particles, dried at 50 °C, and the implants were sterilised in an autoclave.

4.2.2. Animal surgery

The implants were placed into the trabecular bone of the femoral condyle. Each animal received 4 implants in the lateral and medial condyle of both the left and

right femur. Anaesthesia was induced by intravenous pentobarbital, 25mg/kg, and each animal was also given 0.5 mg of atropine. After orotracheal intubation, anaesthesia was maintained by ethrane solution, 2-3 %, through a constant volume ventilator. For the insertion of the implants, a longitudinal incision was made in the medial and lateral surface of both the left-side and right-side femur after the operation area was shaved and disinfected with povidone iodine. After exposure of the femoral condyle, pilot holes were drilled in the trabecular bone. These holes were gradually widened to the final diameter of the implants. The bone preparation was performed with a very gentle surgical technique and with continuous internal cooling. Following the press-fit insertion of the implants, the soft tissues were closed in separate layers with resorbable Vicryl® 2-0 sutures. For prophylaxis, antibiotic Albipen® was administered for 3 days, starting 1 h postoperatively. A total of 48 implants were placed: 12 Ti, 12 HA-coated, 12 HAHT-coated, and 12 FA-coated implants. The implants were placed according to a balanced split-plot design. Three months after insertion of the implants, the animals were sacrificed, using an overdose of Nembutal®. The femoral condyles were excised and preserved in a buffered 10 % formalin solution.

4.2.3. Preparation for Dual X-Ray Absorptiometry.

After fixation, the specimens were dehydrated with an alcohol series. For standardised orientation during sawing and scanning, one end of a wooden cocktail stick was placed into the cylindrical opening of each implant (Figure 4.1). Finally, the specimens were embedded in polymethylmetacrylate (PMMA), a tissue equivalent material. Using a Conrad® saw, the specimens were sectioned in parallel to the long axis of the cocktail stick to a final thickness of 13 mm. The average surface of the section was 2 by 3 cm (Figure 4.1). The sawing section was chosen in such a way that the X-ray beam in the Dual Energy X-Ray Absorptiometry could have access to the trabecular bone structure.

For reasons of comparison, the medial and lateral part of the femoral epicondyle of untreated goats also had to be measured. For this part of the study, we used the excised left- and right-side femoral epicondyles of three female goats (A, B,

and C). These condyles were sectioned in the sagittal plane with 13 mm sections, like the implant specimens (Figure 4.2).

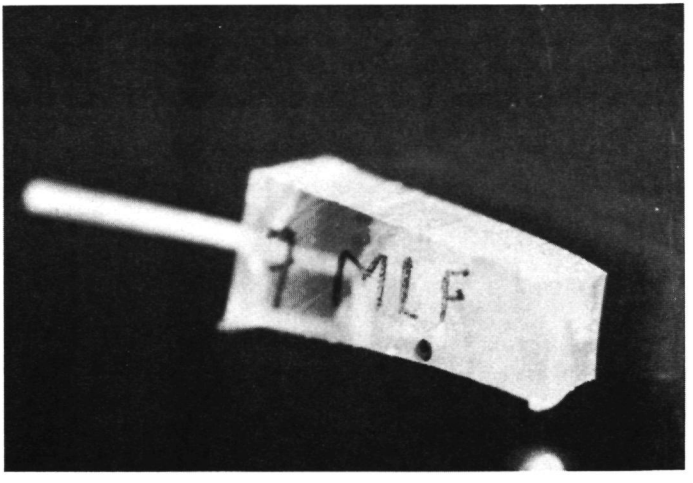


Figure 4.1: The sawn-off polmethylmetacrylate block with a thickness of 13 mm, containing an implant. A cocktail stick is placed into the cylindrical opening of the implant.

4.2.4. Dual X-Ray Absorptiometry scanning procedures

The Dual X-Ray Absorptiometric measurements were made with a Hologic QDR®-1000 bone densitometer (Hologic Inc., Waltham, MA, USA.). We used a source collimator with a diameter of 1 mm and high-resolution software. The line spacing and point resolution were both 0.0127 cm. To define the BMD around and at various distances from the implants, seven regions of interest (R1, R2,.....,R7) were measured. These regions were situated in the trabecular bone structure parallel to the long axis of the implant (Figure 4.3). Region R1 was close to the implant at a distance of 0.0127 cm, Region R2 was adjacent to the first region, and then immediately came region R3. To compare these regions with bone density at a distance from the implants, in the same sequence, control region R4, R5, and R6 were positioned at random in the condyle. Region 7 was situated at a distance of 16 pixels cranial of the implant. Each region had a

length of 45 pixels and a width of 5 pixels. Each sawn-off block was scanned 5 times using the autoscan program. The scans were analysed twice with the regions R1, R2, and R3, all causally (Figure 4.3) of the implant and then cranially of the implant (Figure 4.4). The other regions were kept at their original positions in the second series of the analysis.

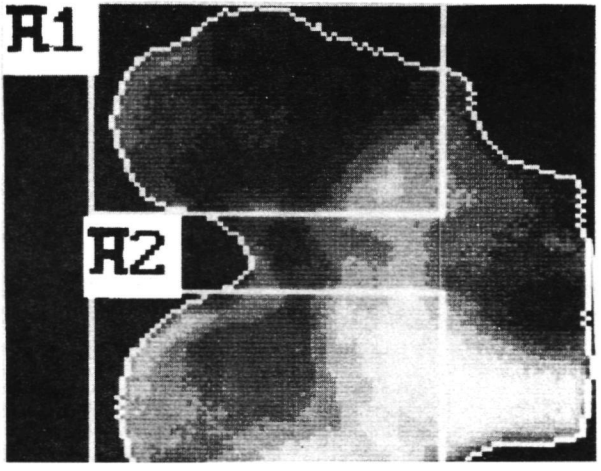


Figure 4.2: Scan of the non implanted sliced femoral epicondyle of goat A, B, and C. The Region Of Interest (ROI) A1 and A2 are respectively situated in the lateral and medial part.

To make inquiries about the BMD in the total area of the 6 untreated control blocks from three animals without any implants we had to use the lumbar spine software with variable resolution and a collimator of 2.3 mm because of the size of the sliced epicondyle. These measurements were performed using the same Hologic® bone densitometer. The line spacing was 0.0470 cm and the point resolution was 0.0481 cm. The size of the lateral Region A1 and the medial Region A2 were both 69 - 47 pixels (Figure 4.2). The samples were immersed in a tissue-equivalent material (5 cm of water). To determine the differences in density in the central direction (see arrow Figure 4.5) as well as in the cranial direction (see arrows Figure 4.6) of the sliced epicondyle, we used the regions

P1 through P6, sized 7 by 27 pixels (Figure 4.5 and Figure 4.6). BMD, as measured with Dual Energy X-Ray Absorptiometry, is usually expressed as grams per surface area (g/cm^2). This definition of 'density' is used by the manufacturer because it consists of a projected BMD of cortical as well as trabecular bone. In the present investigation, all measurements were performed only in slices of trabecular bone, with a thickness of 13 mm. Consequently, density was defined as grams per volume unit (g/cm^3).

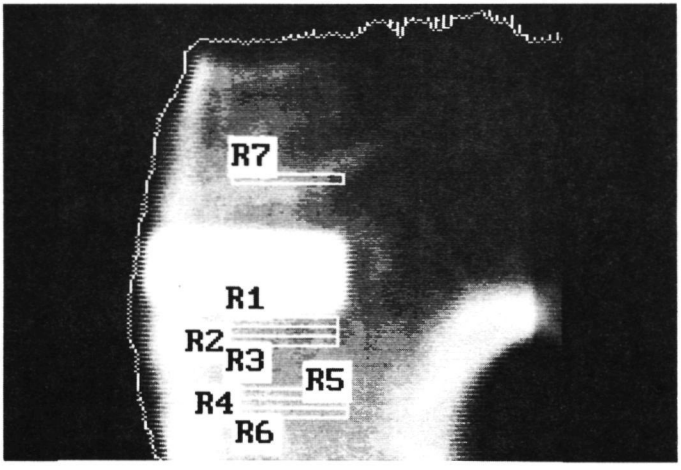


Figure 4.3: Scan of the sawn-off block containing an implant. The ROI's R1-R3 are situated caudal from the implant. Region R7 is at a distance of 16 pixels. The ROI's R4-R6 are positioned anywhere in the condyle.

4.2.5. Histological procedures

After scanning the blocks, thin sections (10 µm) were prepared using a modified diamond-blade sawing-microtome technique. The sections were made perpendicular to the long axis of the implants, stained with methyl blue and basic fuchsin, and examined by light microscopy. To define the percentage of bone contact, the microscopic images were projected onto a monitor by a video camera coupled to the light microscope. The percentage of bone contact was

defined as the length of the interfacial area where there was direct bone apposition divided by the total implant perimeter multiplied by 100. Three sections were used from each implant for bone contact analysis. The results presented are the average value of these three measurements.

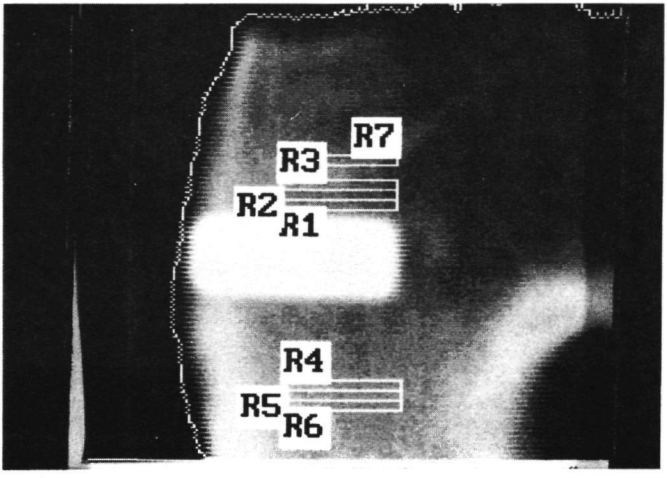


Figure 4.4: The same like Figure 3, but the ROI's R1-R3 are situated now cranial from the implant.

4.3. RESULTS

All animals recovered quickly. One goat had to be excluded 9 days after surgery because of a broken rib. The other animals showed no clinical signs of inflammation or adverse tissue reactions around the implants. Consequently, at the end of the implantation period 44 implant containing specimens were available for evaluation.

Scans taken perpendicularly to the long axis of the implant showed that the implants were actually located in the trabecular bone. Only their coronary parts were surrounded by cortical bone (Figure 4.7).

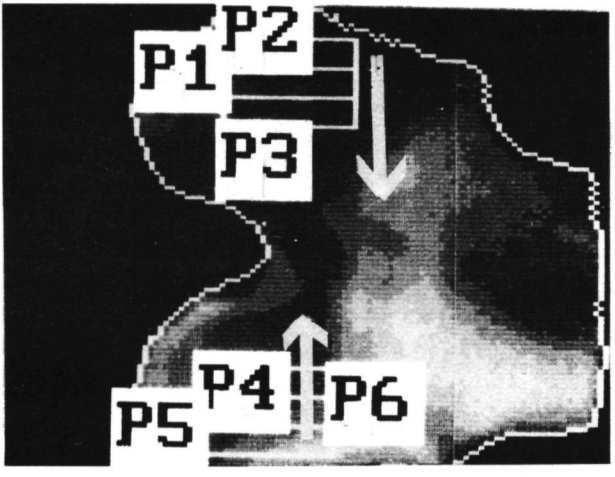


Figure 4.5: Scan of the non-implanted sliced femoral epicondyle. The ROI's P1 through P3 are situated on the lateral side, the ROI's P4 through P6 on the medial side of the epicondyle to central direction (see arrows).

4.3.1. Precision of the measurements

The BMD was calculated as the bone mineral content in g/cm^3. Every block was measured five times, and the precessions were expressed as coefficients of variation for every region. The averages of these coefficients of variation for regions R1 through R3 (close to the implants) were, respectively, 0.44 %, 0.40 %, and 0.40 %. For the regions at distance from the implants (R4 through R7), the coefficients of variation were, respectively, 0.49 %, 0.43 %, 0.47 %, and 0.44 %.

4.3.2. The bone mineral density by implant location

Statistical analysis by Student's t-test showed for implants inserted in both the medial and the lateral condyle significantly higher bone mineral density of region R1 as compared to regions R2 through R7 ($p<0.001$, see Table 4.1).

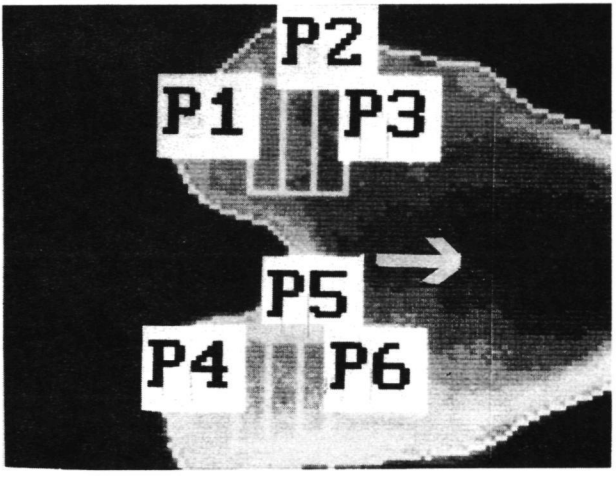


Figure 4.6: Scan of the non-implanted sliced femoral epicondyle. The ROI's P1 through P3 are situated on the lateral side, the ROI's P4 through P6 on the medial side of the epicondyle to cranial direction (see arrow).

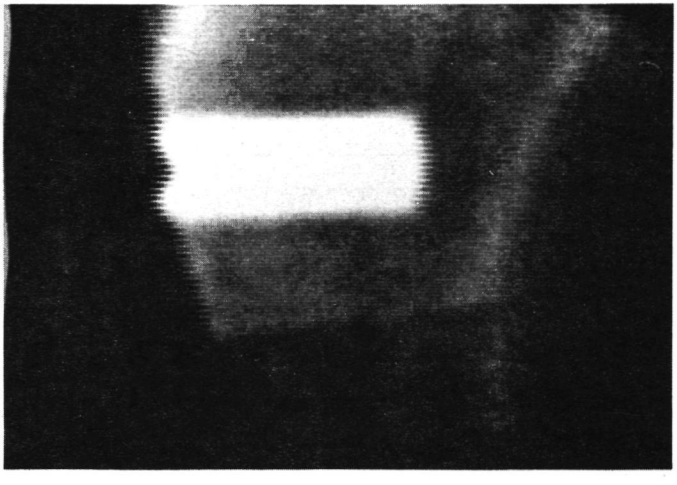


Figure 4.7: Scan of a sawn-off block containing an implant located in the trabecular bone. Only the coronary part is surrounded by cortical bone.

The BMDs of regions R1, R2, R3, and R7 located in the medial side of the condyle exhibited a higher bone density than did the corresponding regions in the lateral side. However, regions R4 through R6 showed the reverse.

Table 4.1: Means, standard deviations, and ranges of the bone mineral densities in g/cm^3 in regions R1-R7, for location in 44 implants. The number of measurements per region was 5.

Region N=5	Location	
	Medial BMD g/cm^3	Lateral BMD g/cm^3
R1	0.6369 ± 0.0905	0.5882 ± 0.0842
R2	0.6146 ± 0.0935	0.5679 ± 0.0843
R3	0.6122 ± 0.0933	0.5665 ± 0.0882
R4	0.5405 ± 0.1231	0.5627 ± 0.0878
R5	0.5477 ± 0.1209	0.5655 ± 0.0921
R6	0.5543 ± 0.1201	0.5648 ± 0.0920
R7	0.5788 ± 0.0875	0.5620 ± 0.0837
RANGE	0.8820 - 0.2750	0.8340 - 0.3694

4.3.3. The bone mineral density by implant type

Table 4.2 shows the BMD for region R1 related to implant type and location. Statistical analysis with an ANOVA and the Newman-Keuls Multiple Comparison procedure demonstrated that the BMD for region R1 of the various implants was significantly higher ($p < 0.001$) in the medial than in the lateral side of the condyle. Further evaluation also revealed that the bone around the Ti implant in the medial side had a significantly higher density ($p < 0.002$) in comparison to all the other types of implants inserted in the same side.

4.3.4. The bone density of the untreated epicondyles

The BMD results for the medial and lateral parts of the left and right untreated femoral condyles are given in Table 4.3. Descriptive statistical analysis indicated a higher BMD in the medial than in the lateral part for both the left and right epicondyles of the three goats.

Figure 4.8 shows the differences in BMD from lateral to central over regions P1-P3 in the lateral and over regions P4-P6 in the medial side of the 6 sliced epicondyles. As indicated, the density in the medial side declined over the three regions. In the lateral part the reverse was observed. The differences in BMD from caudal to cranial over region P1-P3 in the lateral and over the regions P4-P6 in the medial side of the untreated epicondyle are presented in Figure 4.9. In the medial part, no clear difference in density between the 3 regions was observed. However, in the lateral side it tended to decrease.

Table 4.2: Means, standard deviations, and ranges of bone mineral densities in g/cm^3 for region R1, pertaining to the various implants and locations.

Implant	Location	
	Medial BMD g/cm^3	Lateral BMD g/cm^3
FA	0.6024 ± 0.0725	0.5854 ± 0.0455
HAHT	0.5969 ± 0.0727	0.5797 ± 0.1228
HA	0.6326 ± 0.0892	0.6016 ± 0.0848
Ti	0.7050 ± 0.0801	0.5784 ± 0.0500
RANGE	0.4569 - 0.8820	0.3905 - 0.8340

Table 4.3: Bone mineral densities in g/cm^3 for the medial and lateral part for the left (L) and right (R) sectioned femoral epicondyles of goats A, B, and C.

Goat Epicondyle	Location	
	Medial BMD g/cm^3	Lateral BMD g/cm^3
AL	0.503	0.432
AR	0.541	0.447
BL	0.566	0.463
BR	0.532	0.477
CL	0.654	0.469
CR	0.602	0.489

4.3.5. Histological analysis of bone contact

The histomorphometrical analysis of the bone reaction around the implants demonstrated a variation of up to 20 % in the amount of bone contact between the three sections of each implant used.

Table 4.4 gives all the percentages of bone contact for the various implants and implantation sites. An ANOVA and a multiple comparison procedure (Newman-Keuls) revealed a significant difference in bone contact between implants inserted in the medial and lateral condyles ($p < 0.005$).

Also the bone contact of the Ca-P-coated implants was significantly higher ($p < 0.001$) in comparison with the non-coated implants. No significant difference was noted between the FA-, HA-, and HAHT-coated implants.

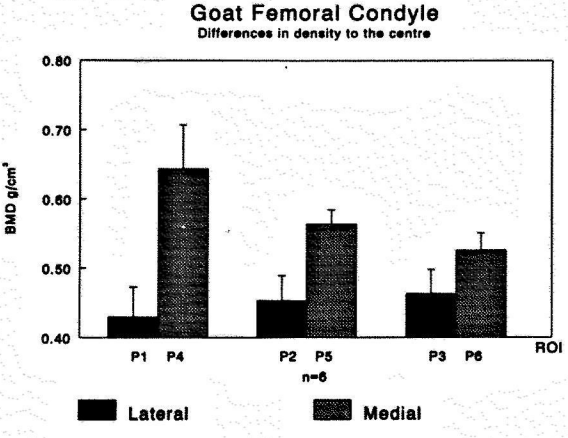


Figure 4.8: The bone mineral densities to the centre of the ROI's P1-P3 in the lateral side and of the ROI's P4-P6 in medial side of the femoral epicondyle.

Table 4.4: Means and standard deviations of bone apposition (%) for the various implants for the two implantation sites. The number of implants of each group was 11.

N = 11	FA	HAHT	HA	Ti
Medial	82.36 ± 7.2	80.58 ± 10.7	84. 34 ± 5.5	60.82 ± 12.7
Lateral	72.8 ± 10	76.98 ± 8.5	74.03 ± 11	49.34 ± 21.4
Mean	78.4 ± 9.1	79.1 ± 8.2	78.2 ± 9.4	56.8 ± 16.9

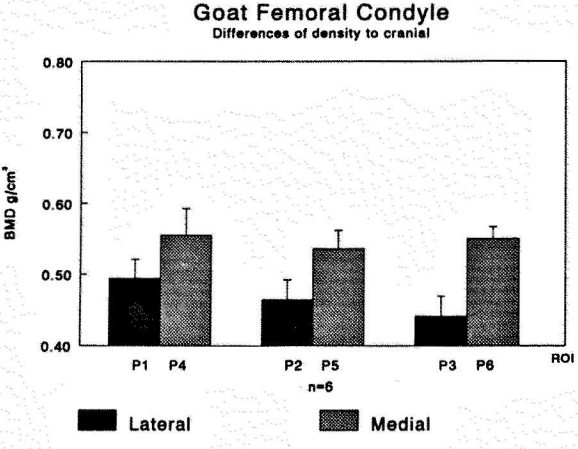


Figure 4.9: The bone mineral densities to cranial of the ROI's P1-P3 in the lateral side and of the ROI's P4-P6 in medial side of the femoral epicondyle.

4.4. DISCUSSION

Repeated analysis showed that the accuracy of the measurements, with a mean coefficient of variation of 0.44 %, is satisfactory.

The precision of the measurements in the excised untreated epicondyles of goats are very similar to previous studies with the lumbar spine protocol by

Kaymakci and Wark [9], and Corten *et al.* [10]. They found a precision of about 0.5 %.

In view of the locations from which the samples were taken, the huge differences between the standard deviations are most probably due to the extremely large range in density between the regions. In addition, the medial regions 1, 2, and 3 had a significantly higher density than did the lateral regions 1, 2, and 3. The reference regions 4, 5, and 6 showed the opposite. These regions were placed towards the centre of the epicondyle relative to regions 1, 2, and 3 due to space limitations at both sides of the implants. This phenomenon can be explained by the results of the BMD measurements in the untreated epicondyles. In these arbitrarily chosen epicondyles, the BMD in the medial compartment was also different from the lateral compartment. This difference is probably due to a difference in load bearing, which influences bone turnover. Studies by Søballe [1] of implants placed in the epicondyle of Labrador dogs showed a similar pattern of distribution of bone density. Consequently, it can be concluded that the BMD around implants depends on the location of the implant. This conclusion is supported by the histological findings which show that the amount in bone apposition was also significantly higher near the implants inserted in the medial compared to those in the lateral sides. Combination of these findings confirms again that, in addition to original bone quality and used implant material, the amount of bone apposition to implants is also determined by biomechanical forces. Moreover, the higher densities in all the regions (R1) close to the implants indicate that there is a strong remodelling activity at distances of approximately 127 µm up to 600 µm (1-5 pixels) from the implants. This phenomenon could be due to the surgery. These effects are still discernible three months after implantation. However, the possibility of a persisting bone reaction being influenced by the type of implant material cannot be excluded. This hypothesis is supported by the lower percentage of bone apposition to the non-coated Ti implant and the higher mineral bone density in region R1 around these implants inserted in the medial epicondyle. The final bone reaction to an implant will be determined by the degree of compatibility and integration of the

inserted implant under certain defined conditions. Consequently, less than ideal bone biocompatibility will result in reduced integration of the implant in the stress transferring system of the surrounding bone trabeculae. This implies that, similar to the soft tissue situation, the implant will act as a constant mechanical stimulus [11]. This trauma will result not only in fibrous encapsulation of the implant but also in more bone turnover around the implant.

4.5. CONCLUSION

The present study shows that two variables - the implant biocompatibility and loading conditions - play primary roles in the amount of bone apposition around implants. Further, we conclude that, along with histological analysis, Dual Energy X-Ray Absorptiometry appears to be an excellent tool in establishing these reactions. These results confirm the need for more research and the development of a device for *in vivo* clinical measurements in implant dentistry.

REFERENCES

1. **K. Søballe** , "Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs.", Acta Orthopaed. Scand. Supplementum no. 255, 64 (1993).
2. **R.M. Meffert**, B. Langer, M.E. Fritz , "Dental Implants: A Review." J. Periodontol., 63, 859-870 (1992).
3. **H. Caulier**, J.P.C.M. van der Waerden , Y.C.G. Paquay , J.G.C. Wolke, W. Kalk, I. Naert , J.A.Jansen, "The effect of calcium-phosphate (Ca-P) coatings on trabecular bone response; A histological study." J. Biomed. Mater. Res., 29, 1061-1069 (1995).
4. **C.C. Jr. Johnston**, C.W. Slemenda, L.J. Melton, "Clinical use of bone densitometry.", New Engl. J. of Med. , 324, 1105-1109 (1991).
5. **B.J. Kiratli**, J.P. Heiner, A.A. McBeath, M.A. Wilson, "Determination of bone mineral density by Dual X-Ray Absorptiometry in patients with uncemented total hip arthroplasty.", J. of Orthop. Res., 10, 836-844 (1992).
6. **R. Mosheiff**, B.Y. Klein, I. Leichter, G. Chaimsky, A. Nyska, A. Peyser, D. Segal, "Use of Dual Energy X-Ray Absorptiometry (DXA) to follow mineral content changes in small ceramic implants in rats.", Biomaterials, 3, 462-466, (1991).

7. **D.J. Kilgus**, E.E. Shimoaoka, J.S. Tipton, R.W. Eberle, "Dual-Energy X-Ray Absorptiometry measurement of bone mineral density around porous-coated cementless femoral implants.", J. Bone Joint Surg. (Br), 75-b, 279-87 (1993).
8. **M. Markel**, F. Gottsauner-Wolf, J. Bogdanske, H. Wahner, E. Chao, "Dual Energy X-Ray absoptiometry of implanted femora after cemented and press-fit total hip arthoplasty in a canine model." J. of Orthopaed. Res., 11, 452-456 (1993).
9. **B. Kaymakci**, and J.D. Wark, "Precise accurate mineral measurements of excised sheep bones using X-ray densitometry." Bone and Mineral., 25, 231-246 (1994).
10. **F.G.A. Corten**, M.A. van 't Hof, W.C.A.M. Buijs, P. Hoppenbrouwers, W. Kalk, F.H.M. Corstens, "Measurements of mandibular bone density ex vivo and *in vivo* by Dual-Energy X-Ray Absorptiometry." Archs oral Biol., 38, 215-219 (1993).
11. **D. Williams**, Concise Encyclopedia of Medical & Dental Materials, 2nd edn. page 51-59, Pergamon Press Oxford (1990).

Chapter 5

ANIMAL STUDY ON BONE BEHAVIOUR TO CA-P-COATED IMPLANTS: INFLUENCE OF IMPLANT LOCATION

H. Caulier[1, 2], T. Hayakawa[2, 4], I. Naert[1], J.P.C.M. van der Waerden[2], J.G.C. Wolke[2, 3], J.A. Jansen[2]

[1] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[2] Department Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[3] Department of Biomaterials, University of Leiden, The Netherlands

[4] Department of Dental Materials, School of Dentistry, Nihon University,Chiba, Japan

5.1. INTRODUCTION

Several clinical studies have demonstrated that areas of the jaws with low bone density, such as the maxilla, offer significantly lower success rates than do areas of denser structure [1-3]. Therefore, several attempts have been made to improve implant anchorage in poor bone conditions, such as the creation of porous implant surfaces [4] and the deposition of Ca-P coatings on the implant surface [5 (see chapter 3)-10]. On basis of these results, it was concluded that Ca-P coatings, like hydroxyapatite and fluorapatite coatings, result in a more rapid initial bone response and a better bone adaptation than is the case with non-coated controls.

In addition to the biological aspect of Ca-P-coated implants, the absence of implant mobility is a mechanical requirement for long-term fixation of implants [11]. This can be achieved by an implant geometry that maximizes bone contact with the implant or by designs that allow bone ingrowth.

In view of these findings, it can be hypothesized that the newly formed bone on the implant surface is the result of biological and biomechanical processes that take place at the interface between bone tissue and the implant surface. Still, most implant studies involve locations that are subject to stress and strain. Consequently, for the objective testing of an implant material, research should be done in mainly load-free situations to exclude the influence of stress and strain on the remodeling processes of bone tissue at the implant-bone interface [12, 13].

The aim of the present study is to compare four different materials installed in the trabecular bone of the mandibular corner of goats. In the literature [14], this implantation region is described as a neutral area, since load transmission is mainly confined to the thin layer of cortical bone whilst the contact area between the implant and the cancellous bone is virtually unloaded.

5.2. MATERIALS AND METHODS

5.2.1. Implant materials

Forty-eight cylindrical titanium alloy implants (Ti6Al4V) were grit-blasted with Al_2O_3 (RA = 4-5 μm). After grit blasting, thirty-six of the implants were coated with a Ca-P plasma-spray coating [15, 5 (see chapter 3)], approximately 55-60 μm thick. The following coatings were deposited (see Figure 5.1):

- hydroxyapatite (HA)
- hydroxyapatite subsequently subjected to a heat-treatment (650° C for 10 minutes) (HAHT)
- fluorapatite (FA)

The coating characteristics are described in chapter 3, section 2.1.

Non-coated titanium implants served as controls. All the implants had a final diameter of 4 mm and a length of 5 mm. After plasmaspraying, the implants were cleaned ultrasonically in 100 % ethanol and sterilized in an autoclave.

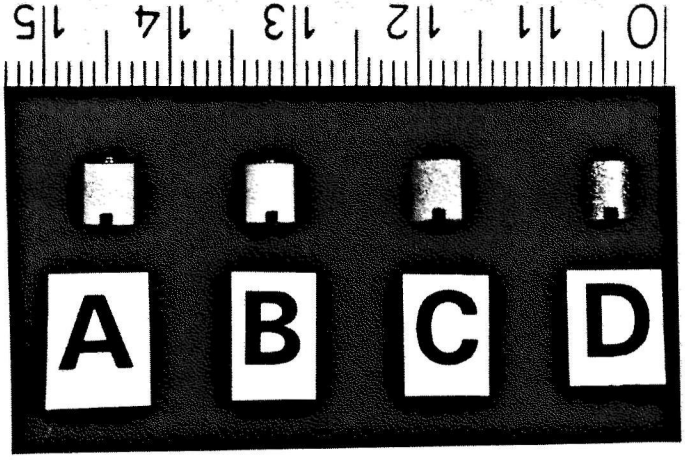


Figure 5.1: Photograph of the four different implant types. A, B, C: Ca-P-coated implants; D: non-coated titanium implant.

5.2.2. Experimental animal design and surgical technique

Twelve female Saane goats with an average weight between 50 and 80 kg and an average age of 30 months were used. The animals were kept in quarantine for at least four weeks prior to the experiment and tested for CAE/CL arthritis. In each goat, all the different implant types were installed in the mandible, two in the left and two in the right corner. The 48 implants were placed according to a balanced split-plot design; 12 HA-coated, 12 HAHT-coated and 12 FA-coated and 12 Ti.

The surgery was performed under general anesthesia induced by intravenous penthobarbital 25 mg/kg and atropine 0.5 mg/animal. After oro-tracheal intubation, anesthesia was maintained by ethrane 2-3 % through a constant volume ventilator.

The animals were immobilized on the back for the insertion of the implants and the mandibles were shaved, washed and disinfected with povidone iodine. The mandible corner was palpated and a longitudinal incision was made parallel to the direction of the masseter muscle. The bone was exposed by blunt dissection of the masseter muscle. Using a drill guide, two pilot holes were drilled at a fixed distance of 2 cm starting from the mandibular corner on the line connecting that corner to the lateral eye corner and half a centimeter perpendicular to that line each (Figure 5.2). The holes were gradually widened to the final diameter of the implants to ensure firm fixation of the implants. The bone preparation was performed using a very gentle surgical technique and continuous internal cooling. After press-fit insertion of the implants, the soft tissues were closed in separate layers using resorbable sutures (Vicryl® 2-0). To reduce the risk of peroperative infection, prophylactic antibiotic Albipen® was administered for three days starting one hour postoperatively.

5.2.3. Histological procedures

The animals were sacrificed 12 weeks after implant installation using an overdose of Nembutal®. The mandibles with the implants were retrieved, and

excess soft and hard tissue was removed immediately to reduce the samples to smaller specimens. These samples were fixated in 10 % buffered formalin solution. To confirm the position of the implants in the mandibular corner, long-cone radiographs were taken perpendicular to the long-axis of the implants (Figure 5.3). The specimens were dehydrated with an alcohol series and finally embedded in methylmetacrylate. Thin non-decalcified histological sections of approximately 10 µm thickness were produced with a modified diamond blade microtome [16, 17]. The sections were stained with methylene blue and basic fuchsin and were ground in a horizontal plane, perpendicular to the long axis of the implant.

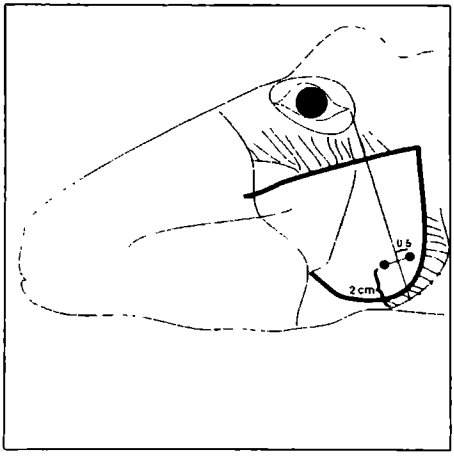


Figure 5.2: Schematic drawing of the location of the implants. The implants were installed at a fixed distance of 2 cm from the mandibular corner on the line connecting that corner to the lateral eye corner, and on half a centimeter perpendicular to that line each.

5.2.4. Histological and histomorphometrical evaluation

Histological and histomorphometrical evaluations were performed to determine the cortical and trabecular bone response to the implants. The qualitative histological analysis consisted of a detailed description of the observed bone

response.

First, for the histomorphometrical analysis, the percentage of bone contact was measured on four sections of each implant using a light microscope connected to a computer equipped with a video and image analysis system (Technical Command Language Image®). Two of these sections were representative of the cortical bone reaction and two for the trabecular bone reaction (Figure 5.4). The amount of bone-implant contact was measured for the total implant perimeter. Finally, the percentage of bone contact, defined as the length of the interfacial area with direct bone-implant apposition, was calculated.

Second, the amount of bone in 2 circular regions of interest (ROI) around the implant was measured (Figure 5.5). These measurements were performed using a stereomicroscope, which was connected to a video camera. A frame grabber with 512 x 512 pixels was used to capture 8-bit grey-level images. One of the two regions was defined in direct contact with the implant at a radial distance of 0.26 mm from the interface (circle A). The other region was determined at 0.61 mm from the implant (circle B). Finally, the amount of bone in the area confined by circle A and in an area called C (C = area of circle A minus the area of circle B) were calculated using the image-analysis program. The amount of bone was quantified as bone amount per $\mu m^2 \times 10^{-3}$. This measurement was performed on the same implant sections as used for the bone contact evaluation.

5.3. RESULTS

One goat had to be sacrificed 9 days after surgery because of a broken rib. The other 11 goats healed uneventfully. At sacrifice, no clinical signs of inflammation or adverse tissue reaction were apparent around the implants. Radiographs taken before embedding of the samples showed that 20 of the 44 retrieved implants were located partially in the mandibular canal.

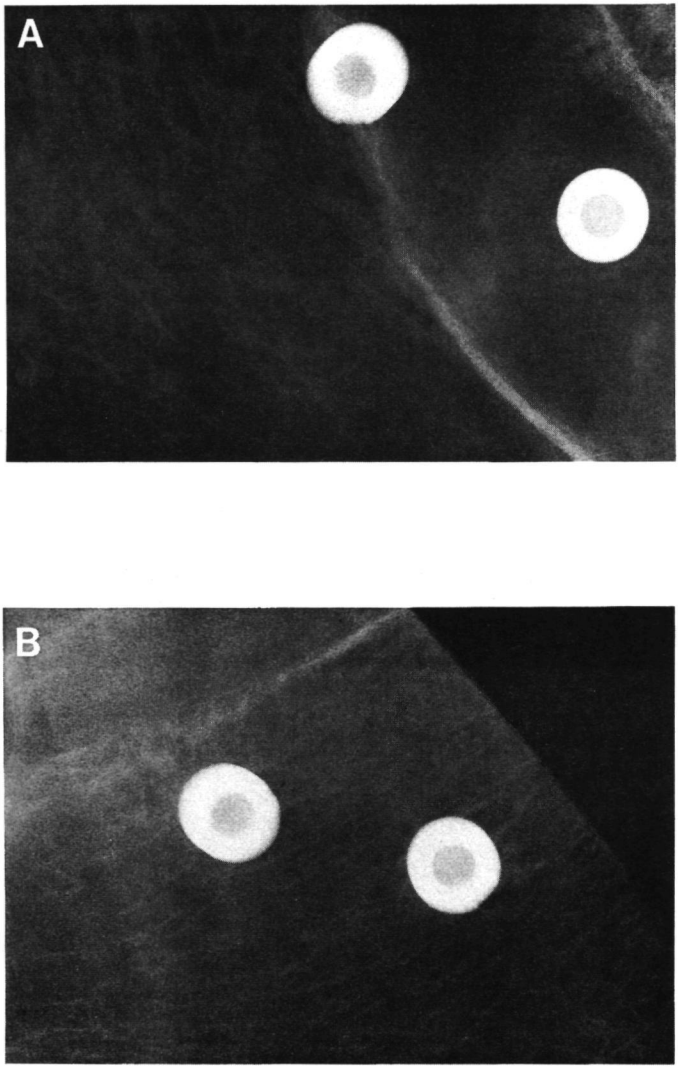


Figure 5.3: A: Radiograph of two implants located in the mandibular canal (C), taken perpendicular to the long axis of the implant.
B: Radiograph of two implants surrounded by trabecular bone, in the vicinity of the mandibular canal (C).

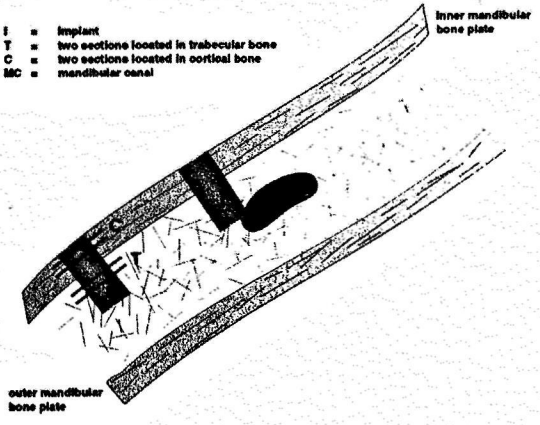


Figure 5.4: Schematic drawing showing that two histological sections were located in the cortical bone (C) of the outer mandibular bone plate, while two sections are located into the trabecular bone (T). It is also visible how the implants are installed in the vicinity of the mandibular canal (MC).

5.3.1. Descriptive histological evaluation

Implants located outside of the mandibular canal:

At the cortical bone level around all the implants, mature bone was observed. This bone was closely laid down to the implant surface without any intervening fibrous tissue interface (Figure 5.6 A).

The trabecular bone reaction was almost identical. Around all the implants, the trabecular bone showed frequently intimate bone-implant contact (Figure 5.6 B). Occasionally, even the implants were completely surrounded by trabecular bone. In areas of bone contact remodeling lacunae with osteoblast were present.

Both in the trabecular and in the cortical bone all coatings showed reduction in thickness. This coating reduction was not uniform and in some areas there was no coating left, while in other areas the coating had not disappeared. Nevertheless, the HA coating reduction was most severe, while the HAHT

coating showed moderate reduction. The FA coating appeared to be very stable.

Samples of implants inside the mandibular canal:

The cortical bone reaction to implants inside the mandibular canal was similar to that of implants installed in a correct position outside the canal (Figure 5.7 A). Because of the bad position inside the canal, it was found that only some implants were inserted completely in the canal. Generally, only a small part of the implant perimeter was in direct contact with the content of the canal. Nevertheless, no adverse tissue reactions were observed in either situation.

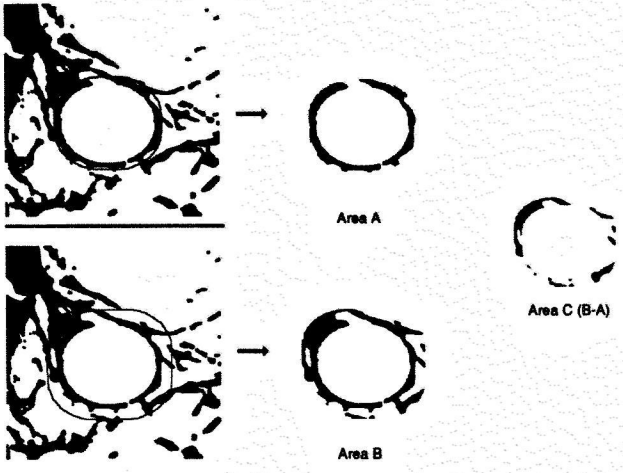


Figure 5.5: Drawing of the regions of interest used for the bone mass measurements.
circle A: region in direct contact with the implant, at a radial distance of 0.26 mm from the interface
circle B: region determined at 0.61 mm from the implant
circle C: amount of bone inside circle B subtracted from the amount of bone inside circle A

The trabecular bone response of the part of the implant located outside the canal was similar to that of the correctly placed implants (Figure 5.7 B). Only on some of the coated implants, a layer of osteoid and occasionally mature

bone was seen on the implant part located in the canal. Evaluation of the coating-reduction pattern revealed the same degree of reduction as seen for the correctly installed implants.

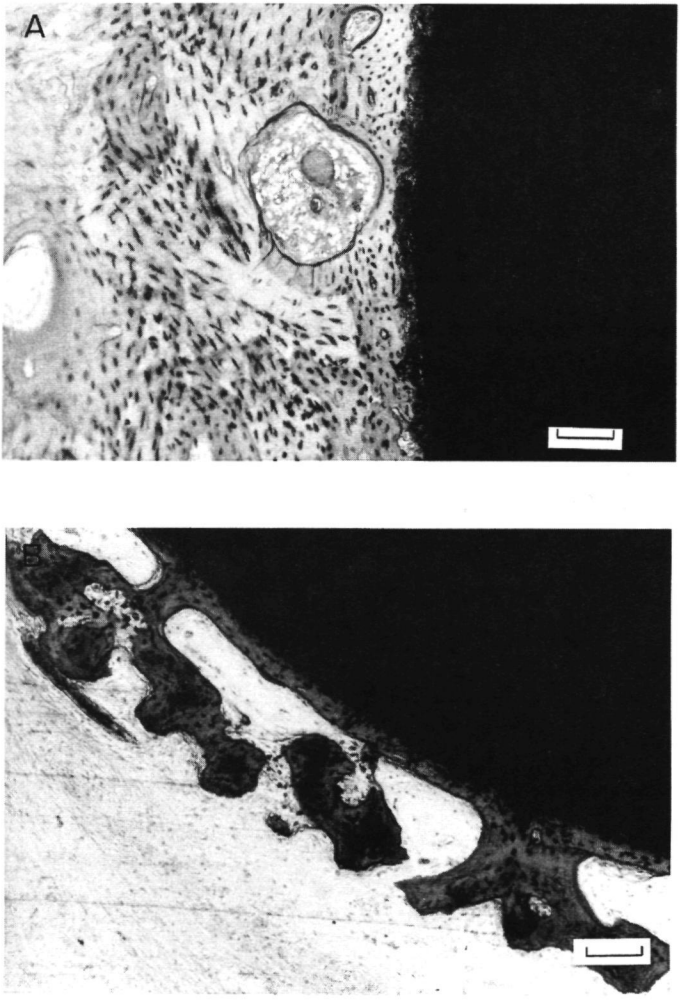


Figure 5.6: Photograph of two implants (I) installed outside of the mandibular canal, surrounded by cortical (A) and trabecular bone (B).
Original magnification: A: 40 x, bar = 73.5 μm
B: 25 x, bar = 117.6 μm

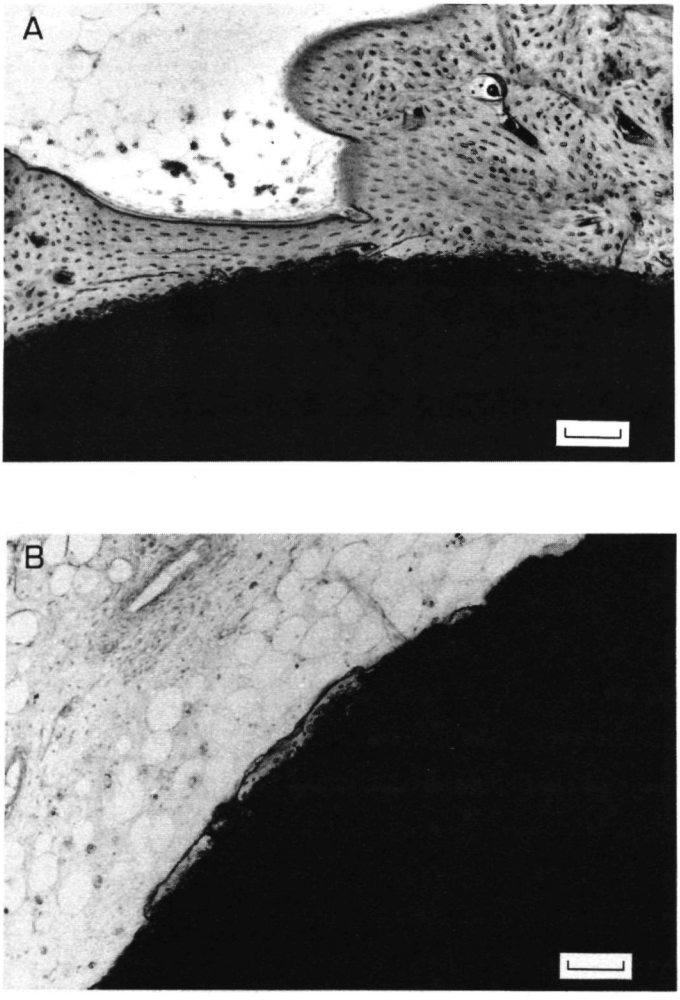


Figure 5.7: Photograph of two implants (I) located inside the mandibular canal, surrounded by cortical (A) and trabecular (B) bone.
Original magnification: A: 25 x, bar = 117.6 μm
B: 25 x, bar = 117.6 μm

5.3.2. Histomorphometrical evaluation

The implants that were located partially inside the mandibular canal were excluded from the histomorphometrical measurements.

Percentage of bone contact

All the cortical and trabecular bone apposition data for the various implant surfaces are given in Tables 5.1 and 5.2. Statistical testing by means of a one-way analysis of variance (ANOVA) and a Tukey multiple comparison procedure revealed no significant difference in percentage of bone contact between the different Ca-P-coated and non-coated implants or between the different Ca-P-coated implants.

Table 5.1: Percentages of bone contact for cortical bone.

Material	Mean % of Bone Contact ± Standard Deviation
Ti	55.4 ± 15.6 (n = 2)
HA	64.3 ± 19 (n = 7)
HAHT	63.6 ± 15.5 (n = 5)
FA	77.9 ± 14.5 (n = 6)

(n = number of implants evaluated)

Table 5.2: Percentages of bone contact for trabecular bone.

Material	Mean % of Bone Contact ± Standard Deviation
Ti	55.7 ± 22.6 (n = 2)
HA	60.9 ± 18.1 (n = 7)
HAHT	65.5 ± 18.5 (n = 6)
FA	71.6 ± 16 (n = 6)

(n = number of implants evaluated)

Bone amount

In Table 5.3, the results are given of the bone amount measurements in areas A and C around the different implant types. One-way analysis of variance (ANOVA) and a Tukey multiple-comparison test revealed no significant differences in cortical and trabecular bone amounts for the non-coated implants with respect to the different coated materials.

Table 5.3: Results of bone amount measurements in $\mu m^2 \times 10^{-3}$.

	Cortical bone				Trabecular bone			
Coating	area A	SD	area C	SD	area A	SD	area C	SD
Ti	9636.0	9900.9	4148.5	272.2	1143.5	733.3	1445.5	618.7
HA	5979.4	3717.3	4514.6	759.0	3248.4	3358.0	2413.3	911.3
HAHT	3047.4	303.7	4092.2	787.9	2394.2	354.1	3045.3	476.2
FA	8308.8	7347.1	4292.8	2505.8	1420.5	780.5	1422.0	918.3

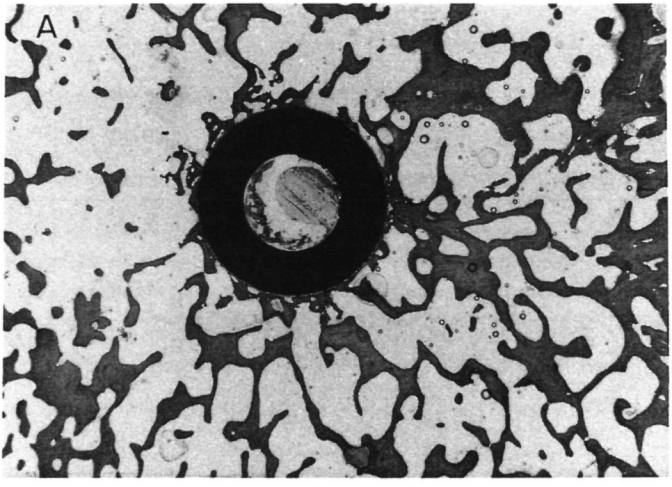
5.4. DISCUSSION

Load-bearing conditions can influence the bone reaction to different implant materials. Consequently, in this study a mainly load-free experimental design was chosen to investigate the bone response to different calcium-phosphate coated and non-coated titanium implants. Radiological and histological investigation revealed that almost half of the number of installed implants were located partially in the mandibular canal, even though a standardized technique was used for the placement of the implants. Consequently, the mandibular corner seems not to be a very suitable and consistent animal model to investigate the bone response to implant materials. To exclude any bias of the bad positioning on the final biological evaluation, all the implants located in the mandibular canal were withdrawn from the histomorphometrical analysis. Consequently, there is a discongruency in the number of specimens for each implant material finally used in the statistical testing procedures.

The results for the cortical bone behavior are very consistent with several other studies performed by Jansen *et al*, [18-20] in rabbits. The data suggest that Ca-P coatings improve the cortical bone contact but statistical testing reveals that this difference is never significant. This phenomenon is due to the relatively high values of the standard deviation, which are probably related to the interanimal differences in the cortical bone quality.

The histomorphometrical measurements also did not show significant differences in trabecular bone reaction between Ca-P-coated implants and non-coated implants. This observation is very surprising in view of our earlier study using the same implants but installed in the femoral condyle of goats [5, see chapter 3]. In that study, we found a significantly lower percentage of trabecular bone in contact with non-coated cylindrical titanium implants in comparison with the Ca-P-coated cylindrical implants.

There are two hypotheses for this finding. First, in the femoral condyle model, as shown by Heimke [12], mechanical factors affect the remodeling process at the bone-implant interface. In the loaded conditions of that model the surface topographical characteristics of the implant play an important role. Second, we know that the surface topography of Ca-P-coated and non-coated grit-blasted titanium implants is not completely similar. Consequently, in mainly load-free conditions, as is the case with the mandibular corner, the influence of the surface topography will be negligible as long as there are no mechanical forces acting on the implant. A second explanation for the difference in bone response between implants inserted in the femoral condyle and the mandibular corner can be the difference in bone morphology. The mandibular bone consists of a thin layer of trabecular bone surrounded by dense cortical bone. The femoral condyle consists mainly of trabecular bone and only a very thin layer of cortical bone. The composition of mandibular bone will result in better initial stability of implants, independent of their surface morphology. In addition, the femoral trabecular bone has a spongy appearance, while the mandibular trabecular bone has a lamellar appearance (Figures 5.8 A and B).



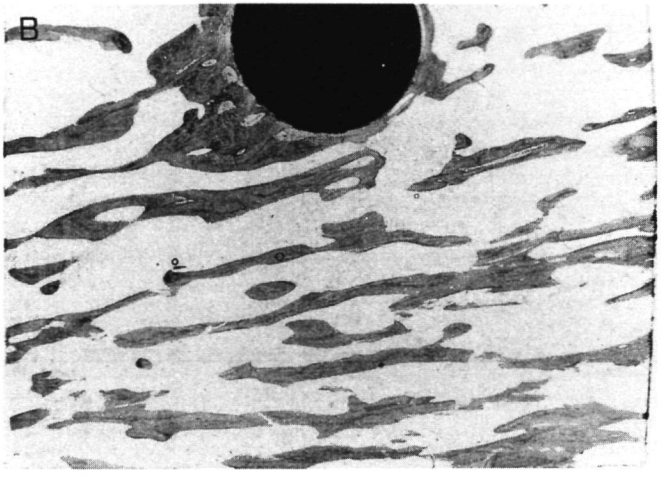


Figure 5.8: Photographs of a cylindrical implant inserted in the trabecular bone of the femoral condyle (A) and of the mandible (B) showing the difference in bone structure between these two different skeletal parts.
A: spongy appearance of the trabecular femoral bone
B: lamellar structure of the trabecular mandibular bone

Histological evaluation of the implants in the mandibular canal showed that only on the surface of some Ca-P-coated implants bone was present on the perimeter section located in the mandibular canal, while bone was never observed on the non-coated implants. We suppose that this bone originated from the bone wall surrounding the canal and subsequently migrated over the implant surface. This observation again demonstrates the osteoconductive properties of Ca-P coatings [21], which provide a scaffold for new bone growth.

The same findings for the coating reduction were observed as in our previous studies [5, 22, see chapter 3 and 6]: the coating reduction did not influence the bone behavior in this 12-week study as we saw no significant differences in bone reaction between the various coatings. The histological sections showed that the bone was in close contact with the implant surface even on places where the coating completely disappeared. Because of similar observations in our previous studies, the coating reduction was not quantified in the present study.

5.5. CONCLUSION

This study shows that the distal corner of the mandible is not an optimal model to install implants because of anatomical restrictions. Moreover, the experiment remained inconclusive about the influence of loading conditions on the bone behavior, probably because of morphological differences in bone structure in the various skeletal parts. Despite these problems, the histological results confirmed at least the bioactive properties of Ca-P coatings.

REFERENCES

1. **R. Adell**, U. Lekholm, B. Rockler, and P.I. Brånemark, "A 15-year study of osseointegrated implants in the treatment of the edentulous jaw", Int. J. Oral Surg., 10, 387-416 (1981).
2. **T. Albrektsson**, B. Bergman, T. Folmer, P. Henry, K. Higuchi, I. Klineberg, W.R. Laney, U. Lekholm, V. Oikarinen, D. van Steenberghe, R.G. Triplett, P.

Worthington, G. Zarb, "A multicenter report on osseointegrated oral implants", J. Prosth. Dent., 60, 75-84 (1988).
3. **R.A. Jaffin** and C.L. Berman, "The excessive loss of Brånemark fixtures in type IV bone: a 5-year analysis", J. Periodontol., 62 ,2-4 (1991).
4. **R.M. Pilliar**, H.U. Cameron, R.P. Welsh, A.G. Binnington, "Radiographic and morphologic studies of load-bearing porous-surfaced structured implants", Clin. Orthop., 156, 249-257 (1981).
5. **H. Caulier**, J.P.C.M. van der Waerden, Y.C.G.J. Paquay, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "Effect of calcium phophate (Ca-P) coatings on trabecular bone response: A histological study", J. Biomed. Mater. Res., 29, 1061-1069 (1995).
6. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, K. de Groot, "Histologic evaluation of the osseous adaptation to titanium and hydroxyapatite-coated titanium implants", J. Biomed. Mater. Res., 25, 973-989 (1991).
7. **W.J.A. Dhert**, C.P.A.T. Klein, J.A. Jansen, E.A. van der Velde, R.C. Vriesde, P.M. Rozing, K. de Groot, "A histological and histomorphometrical investigation of fluorapatite, magnesium whitlockite and hydroxyapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 27, 127-138 (1993).
8. **M. Gottlander**, T. Albrektsson, "Histomorphometric studies of hydroxylapatite-coated and unoated CP titanium implants in bone", Int. J. Oral Maxillofac. Impl., 6, 399-404 (1991).
9. **C.P.A.T. Klein**, P. Patka, H.B.M. van der Lubbe, J.G.C. Wolke, K. de Groot, "Plasma-sprayed coatings of tetracalcium-phosphate, hydroxylapatite and α-TCP on titanium alloy: an interface study", J. Biomed Mater. Res., 25, 53-65 (1991).
10. **M.S. Block**, I.M. Finger, M.G. Fontenot, J.N. Kent, "Loaded hydroxylapatite-coated and grit-blasted titanium implants in dogs", Int. J. Oral Maxillofac. Impl., 4, 219-225 (1989).
11. **G. Heimke**, "Osseointegrated implants", CRC Press, Boca Raton, FL,,10 (1990).
12. **G. Heimke**, P. Griss, E. Werner, G. Jentschura, "The effects of mechanical factors on biocompatibility tests", J. Biomed. Engin., 3, 209-214 (1981).
13. **G. Heimke**, P. Griss, G. Jentschura, E. Werner, "The relevance of histological results for the evaluation of materials for bone replacement", Arch. Orthop. Traumat. Surg., 91, 267-276 (1978)
14. **C. Björk**, "Prediction of mandibular growth rotation", Am. J. Orthod., 55, 585-599 (1969).
15. **K.de Groot**, R.G.T. Geesink, C.P.A.T. Klein, P. Serekian, "Plasma-sprayed coatings of hydroxylapatite", J. Biomed. Mater. Res., 21, 1375-1381 (1987).
16. **H.B.M. van der Lubbe**, C.P.A.T. Klein, K. de Groot, "A simple method for preparing thin histological sections of undecalcified plastic embedded bone with implants", Stain Technol., 63, 171-177 (1988).
17. **C.P.A.T. Klein**, Y.M.H.F. Sauren, W.E. Modderman, J.P.C.M. van der Waerden, "A new saw technique improves preparation of bone sections for light and electron microscopy", J. Appl. Biomater., 5, 369-373 (1994).
18. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, "Histologic investigation of the biologic behavior of different plasma-sprayed coatings in rabbits", J. Biomed. Mater. Res., 27, 603-610, (1993).
19. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, "A histological

evaluation of the effect of hydroxyapatite coatings on interfacial response", J. Mater. Sci., Mater. in Med., 4, 466-470 (1993).

20. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, "Histological and histomorphometrical evaluation of the bone reaction to three different titanium alloy and hydroxyapatite-coated implants", J. Appl Biomater., 4, 213-219 (1993).
21. **K. de Groot**, "Bioceramics of calcium-phosphate", CRC Press, Boca Raton, FL (1983).
22. **H. Caulier**, J.P.C.M. van der Waerden, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "A histological and histomorphometrical evaluation of the application of screw-designed calcium-phosphate (Ca-P) coated implants in the cancellous maxillary bone of the goat", (accepted) J. Biomed. Mater. Res.

A HISTOLOGICAL AND HISTOMORPHOMETRICAL EVALUATION OF THE APPLICATION OF SCREW-DESIGNED CALCIUM-PHOSPHATE (CA-P) COATED IMPLANTS IN THE CANCELLOUS MAXILLARY BONE OF THE GOAT

H. Caulier[1, 2], J.P.C.M. van der Waerden[2], J.G.C. Wolke[2, 3], W. Kalk[2], I. Naert[1], J.A. Jansen[2]

[1] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[2] Department of Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[3] Department of Biomaterials, University of Leiden, The Netherlands

6.1. INTRODUCTION

The success rate for endosseous oral implants seems to be lower in the maxilla. For example, for Brånemark® implants the cumulative success rate for individual implants in the maxilla reaches only 90 % after 4-5 years. In contrast, for implants in the symphyseal area figures above 95 % are reported [1-4]. This poorer performance in the maxilla can be attributed to a less mineralised and less corticalised bone, thus achieving less primary stability at the implant-bone interface during the healing phase [5].

However, the increasing demand for implant supported prostheses encourages the search for new implant surface characteristics and/or geometry's to improve the success rate in the maxilla, especially for bone quality III and IV [6]. To comply with limited and poor bone quality there can be a need for the use of modified implant designs and implants coated with a thin layer of bioactive calcium-phosphate (Ca-P) ceramic. Interest in Ca-P ceramics for endosseous implants is derived from their relative similarity to the mineral phase of bone tissue, i.e. hydroxyapatite (HA) $Ca_{10}(PO4)_6(OH)_2$, octacalcium-phosphate (OCP) $Ca_4H(PO4)_32.5H_2O$ and tricalcium-phosphate (TCP) $Ca_3(PO4)_2$ [7, 8].

According to the current knowledge, the most valuable characteristic of Ca-P ceramics is their ability to become coated with a microscopic layer of bone mineral after insertion into bone tissue [9, 10]. In addition, normal remodelling of these deposited bone layers occurs [11, 12]. In considering these biological advantages, however, it has to be noted that Ca-P ceramics can only conduct bone growth over the implant surface. They are not capable of inducing new bone formation [13].

As a logical consequence of this recognised favourable behaviour, Ca-P materials were used in manufacturing oral implants [14]. However, bulk Ca-P ceramic demonstrates serious mechanical shortcomings. Although the materials have high resistance to compressive forces, they show low tensile and bonding strength. Therefore, implants made of bulk Ca-P cannot be used

in complex loaded situations. To solve this problem it was proposed, in the early 1980's [15] to apply these materials as coating on a metallic surface using a so-called plasma spray process.

Various studies demonstrated a faster and greater bone adaptation to such coated implants [11, 16-21]. The histological results thus obtained, showed significantly higher percentages of bone contact along Ca-P-coated implants compared with non-coated implants. Greater implant stability was also obtained, as confirmed by higher fixation strengths after short and prolonged implantation periods. In addition, some authors[22, 23] suggested that implant surface topography, with the Ca-P coating having a much rougher surface than as-machined titanium implants, is also encouraging bone formation.

Nevertheless, most evaluations of experimental oral implants apply only to high quality cortical bone, whereas the response of less mineralised bone, as is mostly the case in the maxilla, is never thoroughly investigated. In order to get more insight into the response of bone with low density, a goat animal-model was used for installing oral implants. The implants used were self-tapping screw-shaped Brånemark® implants (Nobelpharma, AB, Gothenburg, Sweden). These implants provided not only a larger surface area for bone contact because of the screw threads, but also a good initial stability by means of the self-tapping screw threads of the implant. The rate of clinical success of these implants in the maxilla has been documented [1, 24, 25] to be inferior, compared to the mandible. It was hypothesised that the addition of Ca-P coatings can improve bone healing of implants inserted in low trabecular bone, as present in the maxilla of goats.

6.2. MATERIALS AND METHODS

6.2.1. Implant materials

Sixty-four commercially pure titanium implants with a screw-shaped design (Nobelpharma AB, Gothenburg, Sweden) were used. The implants were self-

tapping, Mk II type, with a diameter of 3.75 mm and a length of 10 mm (see Figure 6.1).

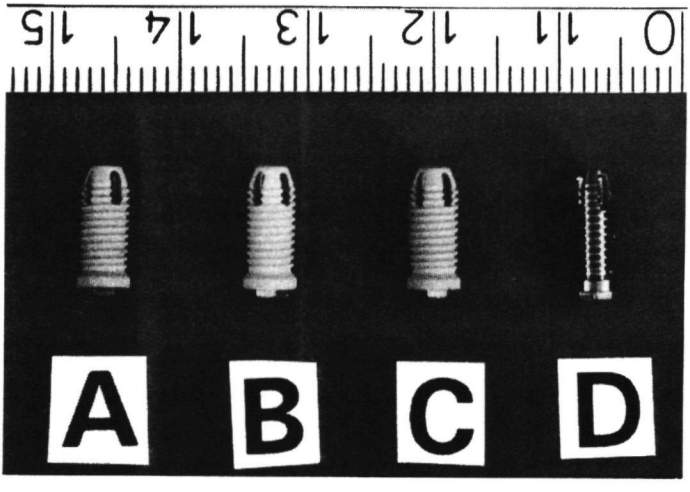


Figure 6.1: Photograph of the four different implant types. A, B, C: Ca-P-coated implants, D: non-coated titanium implant.

All implants that were to be coated were grit-blasted to a roughness of Ra = 4 - 5 µm. Thereafter, the implants were given a calcium-phosphate (Ca-P) coating, approximately 50-60 µm in thickness, using a plasma spray process [15] Three different coatings were produced. The final distribution of the experimental implants was:

* 16 implants coated with fluorapatite (FA)
* 16 implants coated with hydroxyapatite (HA)
* 16 implants coated with hydroxyapatite followed by a heat treatment of 650°C during 10 minutes (HAHT)
* 16 implants, which served as controls and were left non-coated (Ti)

The coatings were characterised by X-ray diffraction (XRD) (Figure 6.2), and infra-red spectroscopy (IR). The analysis revealed a 95 % crystallinity for the

FA-coating, 65 % for the HAHT-coating, and 60 % for the HA-coating [26, see chapter 3] After plasma-spraying, the implants were cleaned ultrasonically in ethanol. Finally, all implants were sterilised in a steam autoclave.

6.2.2. Experimental animal design and surgery.

Sixteen female adult Saane goats, with an average weight of 50-80 kg, were selected. The selection of the experimental animals was determined by having shed their deciduous teeth. Therefore, radiographs were taken to ensure that only permanent teeth were present. The selected animals were kept in quarantine for at least four weeks and tested for CAE/CL-arthritis by taking blood samples.

The first and second maxillary premolars of each of the 16 goats were bilaterally extracted under general anaesthesia. The goats were immobilised on their backs and anaesthesia was induced by intravenous pentobarbital 25 mg/kg and atropine 0.5 mg/animal, and maintained by ethrane 2-3 %, given through an orotracheal tube. After careful extraction, the wounds were closed using resorbable Vicryl® 2-0 sutures. Twelve weeks following tooth removal, the implants were inserted in the edentulous maxillary premolar regions. At that time, the mucosa was exposed under general anaesthesia by a longitudinal incision on the palatal alveolar crest. Two holes were drilled bilaterally with a distance between the holes of at least 7 mm, using the Nobelpharma drilling equipment (Nobelpharma AB, Gothenburg, Sweden).

The holes were undersized with a final drill size of 3.5 mm, to reach a good initial stability of the implants by threading the self-tapping implants (Brånemark®, MK II) into the bone. The bone preparation was performed with a very gentle surgical technique and saline irrigation. Finally, the implants were covered with a cover-screw of pure titanium and the mucoperiosteal flaps were closed with Vicryl® 2-0 sutures.

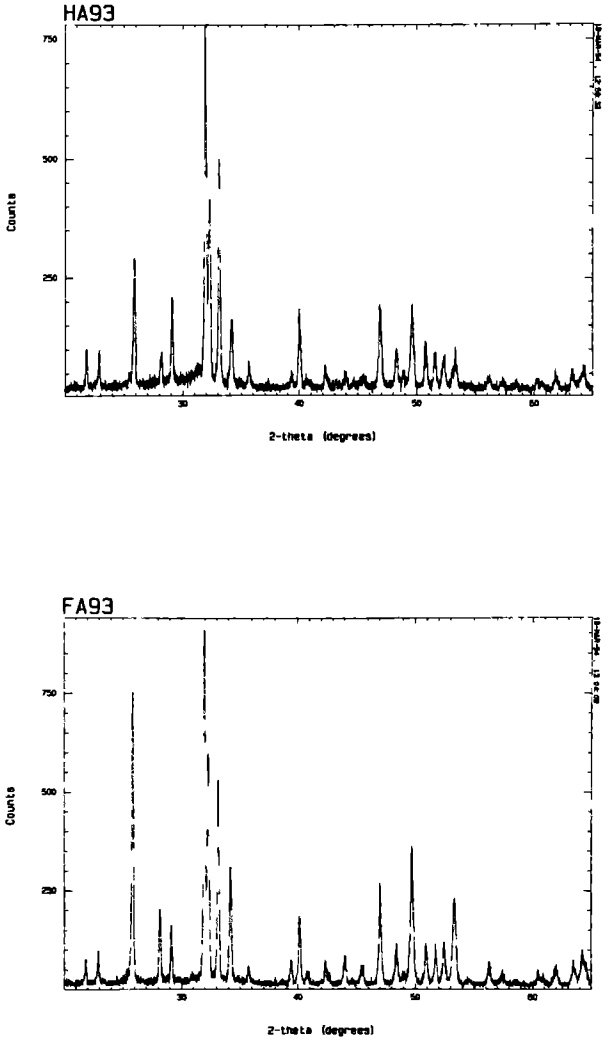


Figure 6.2: XRD-patterns of HA (top) and FA (bottom) plasma-sprayed coatings with the 2 θ in degrees on the x-axis, and the total # counts on the y-axis.

The implants were placed according to a statistically balanced split plot design, in order to compensate for differences in surgical site. Each animal received all

types of implants (FA, HAHT, HA, or Ti). One hour postoperatively, a prophylactic antibiotic therapy (Albipen®) was begun and was given for three days to reduce the peroperative infection risk. The implants were left endosseous for six months to allow healing of the alveolar bone.

At the end of this time, the animals were subjected to a second operation to provide the implants with permucosal abutments. The operation site was disinfected with a 0.1 % chlorhexidine solution. The location of the implants was determined by palpation of the alveolar ridge and the mucosa was opened over the implants with a longitudinal incision. Then, the cover screw of each implant was removed and the abutments were attached. The abutments (Nobelpharma, AB, Gothenburg, Sweden) were also manufactured of pure titanium with a diameter of 4.5 mm and a length of 4 mm. Finally, they were provided with a plastic healing cap and the wounds were closed.

Four months later, the animals were killed by an overdose of Nembutal®.

6.2.3. Histological procedure.

Following the death of the animals, the maxillae were excised as a unit. Subsequently, the left and right implants with the surrounding tissues were removed, divided in small blocks, and fixed in 10 % buffered formalin solution. After dehydration by alcohol series, the implant tissue specimens were embedded in methylmethacrylate. Non-decalcified serial sections of 10 µm thickness were prepared with a modified inner circular saw microtome [27, 28]. The sections were made in a buccopalatal direction parallel to the long axis of the implant surface. The sections were stained with methylene blue and basic fuchsin for evaluation by light microscopy.

6.2.4. Histological and histomorphometrical evaluation.

For both the histological and histomorphometrical measurements it was always the midsection of the serial section of each implant that was evaluated. The histological sections were analysed blind, by the same operator.

The histological evaluation was based on a description of the overall tissue

reaction to the implants.

The histomorphometrical measurements were performed using a Zeiss® light microscope, equipped with a video camera and connected to an Acorn® computer provided with an image analysis software package (TCL®image).

In the selected sections, the following parameters were assessed:

(1) gingiva reaction (Figure 6.3):

(a) The length of the gingival epithelium from the top of the abutment (AB) to the coronal boundary of the connective tissue (CT).

(b) The thickness of the connective tissue from the apical limitation of the gingival epithelium (CT) to the marginal border of the alveolar bone crest (BC).

(2) bone reaction (Figure 6.3):

(a) The first screw thread that showed direct bone contact. As all the implants consisted of 12 screw threads, we numbered the screw threads in ascending order from the most apical (1) towards the most coronal screw thread (12).

(b) Bone contact was defined as a percentage of bone contact, without any intervening soft tissue between bone and implant surface. The measurements were performed along the three best consecutive threads of the implant.

(c) The number of screw threads that showed direct contact with the surrounding bone was calculated.

Measurements 2a and 2c were performed independently of the amount of bone that was in direct contact with the implant surface. All measurements were performed for the buccal as well as for the palatal site of the implant.

(3) coating thickness (Figure 6.4):

The remaining coating thickness was measured along three threads of the buccal and the palatal surface. Therefore, 10 horizontal scan lines perpendicular to the titanium surface were drawn between the respective

coating boundaries. The length of each of these lines was calculated. Finally, these data were classified in 8 groups: 0-7 µm, 8-15 µm, 16-23 µm, 24-31 µm, 32-39 µm, 40-47 µm, 48-55 µm, 56-63 µm.

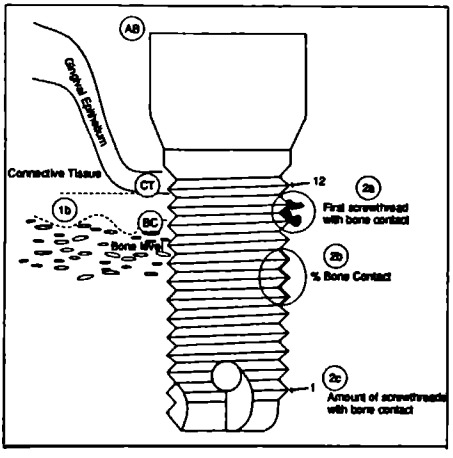


Figure 6.3: Schematic drawing of the histomorphometrical measurements.
The gingival parameters: length of the gingival epithelium (1a: AB-CT), and thickness of the connective tissue (1b: CT-BC) (left).
AB: top of the abutment, CT: boundary of the connective tissue, BC: marginal border of the alveolar bone crest.
The parameters to measure the bone reaction (right):
*2a: first screw thread with bone contact (12 = the most coronal screw thread, 1 = the most apical screw thread)
*2b: percentage of bone contact
*2c: number of screw threads with bone contact

6.3. RESULTS

Of the 64 installed implants 48 implants healed uneventfully. During the endosseous phase (Figure 6.5) 10 of the 64 installed implants were lost. During the permucosal phase another six implants failed. Fourty-two of the 48 Ca/P coated implants were clinically stable (absence of mobility), while only six

of the 16 non-coated titanium implants were still present at the moment of sacrifice. A Chi-square test (Table 6.1) revealed that this difference between the coated and the non-coated implants was significant ($p < 0.001$).

6.3.1. Histological description.

Examination of the histological sections revealed that the bone reaction to the coated implants was relatively uniform regardless of the applied Ca-P coating. Large parts of these implant surfaces showed close bone contact with mature bone, interrupted by areas of newly formed bone. All Ca-P coatings showed signs of reduction in thickness.

The reduction of coating thickness did not interfere with bone contact (Figure 6.6). No cellular activity of multinucleated cells could be observed in the vicinity of the implant surfaces.

The titanium non-coated implants seemed to be surrounded by less bone than the Ca-P-coated implants. Soft tissue or marrow tissue was often interposed between the implant and bone surface. At places where no fibrous layer was present, close bone apposition to the implant surface was observed (Figure 6.7 left and right).

Around all the implants there was a limited downgrowth of the gingival epithelium (Figure 6.8). The epithelium mostly appeared to form a stable junction with the implant surface. Two types of gingival response to the permucosal implant surface could be observed. In none of these sections were collagen fibres found which were oriented perpendicular to the implant surface. The implants displayed no gross inflammatory reaction in the gingival tissue.

6.3.2. Histomorphometrical evaluation.

The three parameters analysed were gingiva reaction, bone reaction and coating thickness. The outcome of these evaluations are given in Table 6.2, Table 6.3 and Figure 6.9. The data as presented, are the average of palatinal and buccal measurements.

Table 6.1: The Chi-square results of the maintained and lost implants.

Implant Material	Number and Percentage of Maintained Implants	Number and Percentage of Lost Implants	Total Numbers and Percentages of Inserted Implants
Ca-P Coated	42	6	48
	65.6 %	9.4 %	75.0 %
Non-Coated	6	10	16
	9.4 %	15.6 %	25.0 %

Degree of freedom = 1, Chi-square = 16.0, Probability < 0.001

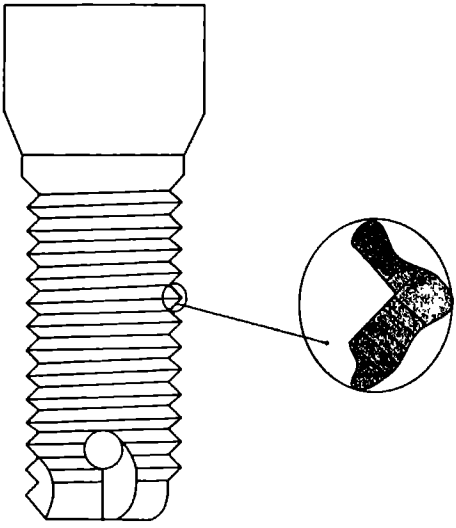


Figure 6.4: Schematic drawing of the measured coating thickness. On three screw threads (buccal and palatal) 10 horizontal scan lines were drawn perpendicular to the implant surface. The length of these lines was calculated to determine the remaining coating thickness.

Statistical testing of the gingiva data, using a one-way analysis of variance (ANOVA) and a multiple Newman-Keuls comparison procedure, showed that only a significant difference existed in connective tissue reaction. The connective tissue layer around the non-coated implants was significantly thicker than around the Ca-P-coated implants ($p = 0.003$).

Statistical testing of the bone data, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman-Keuls), revealed a significant difference ($p < 0.001$) between the non-coated and the Ca-P-coated implants for the first screw thread showing bone contact. The first screw thread was located more coronally for the coated implants. No significant difference was demonstrated between the various Ca-P-coated implants.

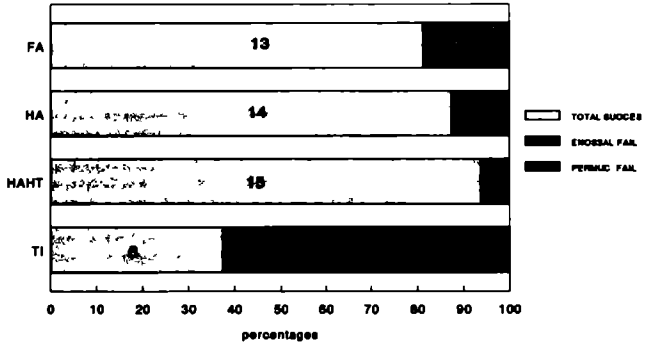


Figure 6.5: Clinical success rates of all the Ca-P coated and non-coated implants. The four bars represent the percentages of total success, endosseous fails, and permucosal fails of the FA-, HA-, HAHT- coated and non-coated titanium implants.

Table 6.2: Histomorphometrical data of the gingiva reaction to the various implant materials.

	Titanium	Hydroxy-apatite	Hydroxy-apatite Heat-Treated	Fluorapatite
Epithelial Length	190 ± 131.7 (n = 6)	285 ± 134 7 (n = 14)	309.7 ± 97.2 (n = 15)	300.2 ± 160 (n = 13)
Connective Tissue Thickness	632 ± 252.2 (n = 6)	366 ± 187 8 (n = 14)	412.6 ± 124.8 (n = 15)	324.3 ± 100.7 (n = 13)

Mean values in mm ± SD, n = number of implants

Figure 6.6: Light micrograph of a HAHT- coated implant showing areas of close bone contact with mature bone interrupted by areas of newly formed bone. Even at places where the coating disappeared (arrow), the bone was in close contact with the implant surface. Original magnification: 25 x, bar = 85 µm.

In addition, a significantly ($p = 0.001$) higher percentage of direct bone contact was found for the various coated implants (HAHT = 63.1 %, HA = 67.4 %, FA = 77.1 %), compared to the titanium controls (26.5 %). The differences among the coated implants were not significant. Statistical testing also showed that a significantly ($p < 0.001$) higher amount of screw threads of the Ca-P-coated implants were in contact with bone, compared to the titanium non-coated implants. Again, the differences among the various Ca-P-coated implants were not significant.

Further, using a simple linear regression test, no correlation could be

demonstrated (r = 0.5) between (1) the length of the connective tissue with the first screw threads showing bone contact, and (2) the length of the connective tissue and the number of screw threads showing bone contact.

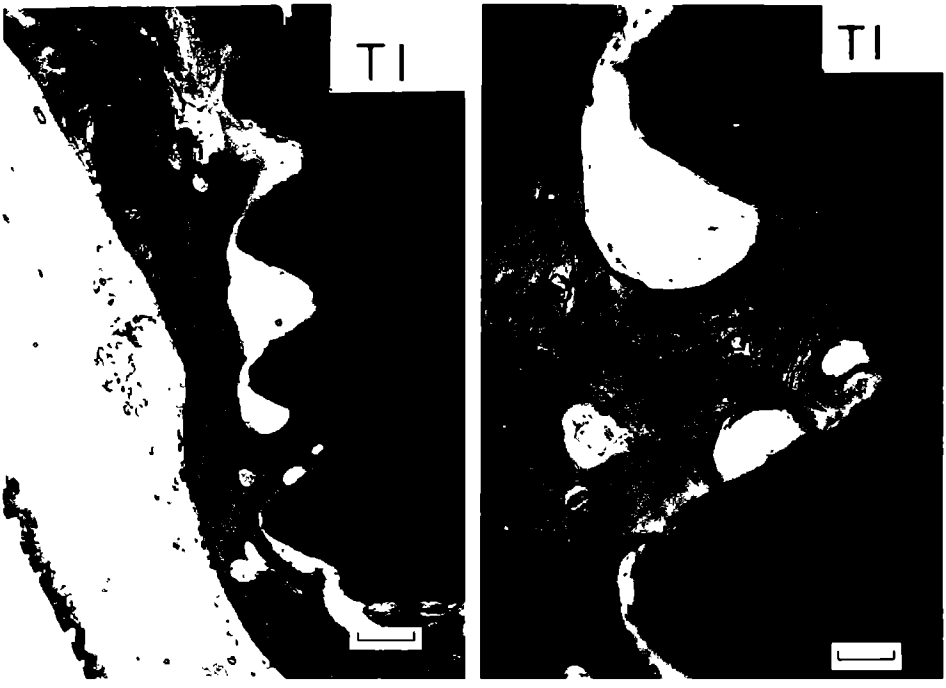


Figure 6.7: Left: light micrograph of a non-coated implant showing the implant surrounded by a fibrous tissue layer. Only in the apical part of the implant is there bone contact with the implant surface. Original magnification: 10 x, bar = 294 µm
Right: Magnification of the 2 apical screw threads of the left radiograph. Close bone apposition can be observed where there is no fibrous tissue present. Original magnification. 40x, bar = 73.5 µm.

The results of the coating thickness measurements indicate that all coatings show reduction in thickness, but also that the reduction is not uniform. In some areas there is no coating left, while in other areas the coating did not

disappear The reduction is most severe for the HA-coated implants The FA-coating seemed to be the most stable one

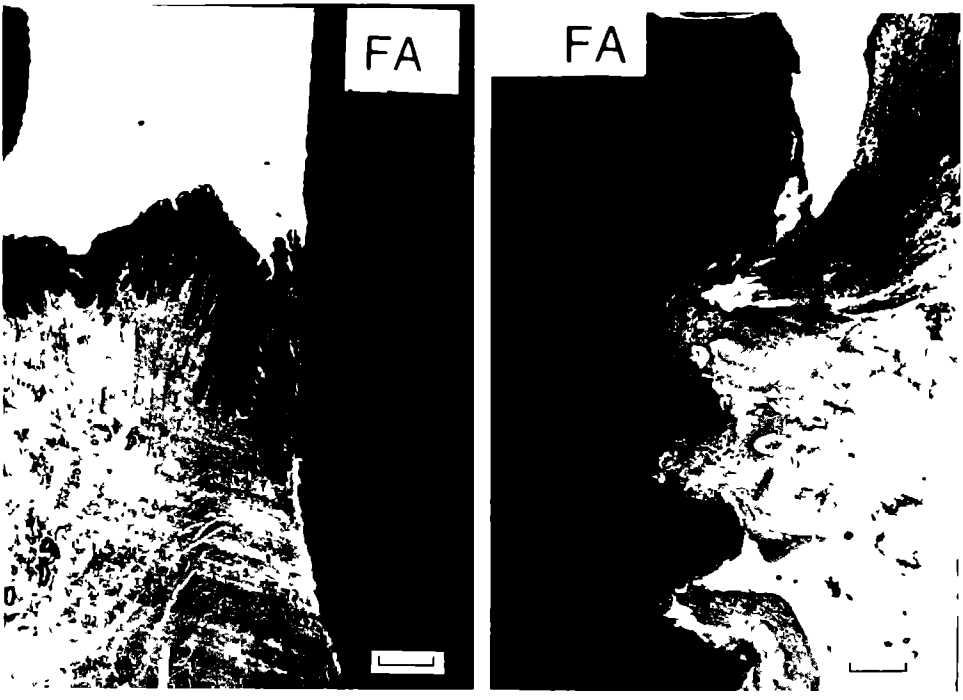


Figure 6.8 Photographs of the same FA-coated implant, the length of the gingival epithelium was confined to the neck of the implant Original magnification 10 x, bar = 294 µm
Two different types of gingival response were present
left On the left photograph the gingival response was characterised by the formation of a gingival epithelium followed by a thick connective tissue layer
right In the right photograph the gingival epithelium was followed by a thin connective tissue layer, immediately followed by the bone level

Table 6.3: Histomorphometrical data of the bone reaction to the various implant materials.

	Titanium	Hydroxy-apatite	Hydroxy-apatite Heat-treated	Fluorapatite
Percentage Bone Contact	26.5 ± 16.2 (n = 6)	67.4 ± 27 (n = 14)	63.1 ± 24.9 (n = 15)	77.1 ± 13 (n = 13)
First Screw thread with Bone Contact	4.3 ± 3.3 (n = 6)	8.2 ± 2.4 (n = 14)	8.0 ± 2.6 (n = 15)	9.6 ± 2.2 (n = 13)
Number of Screw threads with Bone	7 ± 4.4 (n = 6)	13.6 ± 5.9 (n = 14)	12.3 ± 5.7 (n = 15)	15.1 ± 5.6 (n = 13)

Mean values ± SD, n = number of implants

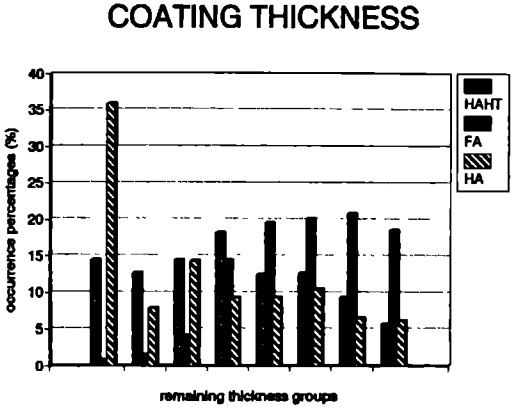


Figure 6.9: Bar diagram containing the results of the coating thickness measurements. The results are classified in 8 groups containing the data of the remaining coating thickness in micrometer from left to right; 0-7; 8-15; 16-23; 24-31; 32-39; 40-47; 48-55; and 56-63 µm (x-axis). On the y-axis the percentages of occurrence are given of the HA-, FA-, and HAHT- coated implants.

6.4. DISCUSSION

The aim of the study was to investigate the trabecular bone response to non-coated and various Ca-P-coated oral implants.

The clinical results revealed that more Ca-P-coated implants were stable than were the non-coated Ti implants. Besides the poorer clinical success of the non-coated Ti implants, this study also demonstrated that the biological bone reaction of the successful non-coated Ti implants was less favourable compared to the coated implants. The coated implants showed a significantly higher percentage of bone in contact with the implants; the first screw thread with bone contact was located more coronally and the amount of screw threads showing bone contact was significantly higher compared to the non-coated implants. These findings confirm the results of other studies [11, 12, 26 (see chapter 3), 29] with Ca-P-coated implants. However, it has to be emphasised that the low clinical success of the non-coated implants, as found in our study, has never been observed in other studies. Indeed, the maxilla of the goat lacks a distinct cortical layer and the implants are exclusively surrounded by cancellous bone. Apparently, these findings show that Ca-P coatings can play an important role during healing and bone ingrowth of oral implants in bone of poor density.

Still, it has to be emphasised that the preceding grit blasting of the implant surface and the irregular surface topography of the coatings can be responsible for the bone response rather than the Ca-P nature of the coatings themselves. This explanation is supported by the findings of Courtney *et al.* [30]. They investigated extracellular matrix production on smooth and rough hydroxyapatite surfaces in cell culture. Matrix production was increased on the rougher surfaces.

Another interesting finding in this study was the serious reduction in coating thickness for the HA- and HAHT- coating. Nevertheless, in this 10-month study, this coating reduction did not influence the trabecular bone behaviour, since we saw no significant difference in the percentage of bone contact for

the three different coatings. The lower degradation rate of fluorapatite coatings has been described earlier [11, 26, see chapter 3]. Still, the final clinical efficacy of such coatings for oral implants needs further investigation. For example, Kangasniemi *et al.* [31] determined in animal experiments the tensile bone-bonding strength of FA- and HA- plasma-sprayed coatings. They observed that after achievement of bone contact, all fracture failures occurred at the coating-titanium interface. On the basis of these findings, they concluded that plasma-sprayed coatings should not be used in load-bearing situations where delamination of the coating would be detrimental to the final implant life-time.

Despite the encouraging results with the Ca-P coatings in this study and the fact that bone was still in close contact with the implant surface at places where the coating completely disappeared, the long term effect of the biodegradation of the coating and what will happen at the bone-implant interface when the coating has disappeared remains a point of concern. Especially, in relation to the grit blasting procedure with Al_2O_3 particles to obtain mechanical retention of the coating. For example, remaining Al_2O_3 particles can change the biocompatibility of the implants. On the other hand, as supposed by Gotfredsen *et al.* [32] a possible solution for this problem is the use of titanium dioxide particles in the blasting procedure.

Further, in the present experiment, no significant difference in the behaviour of the gingival epithelium to the various implant surfaces was observed. These findings confirm the earlier *in vitro* cell studies and *in vivo* animal studies by Jansen *et al.* [33-35]. On the basis of various experiments, they concluded that epithelial cells always behave the same in attachment and growth, independently of the substrate surface conditions. Therefore, it can be supposed that the length of the gingival epithelium is of no importance as a parameter with predictive value for the quality of the permucosal passage and the success of permucosal devices.

On the other hand, the length of the connective tissue seems to be an important parameter. The measured thickness varied significantly between the

coated and non-coated implants. A significantly thicker connective tissue was measured along the Ti implants, while the differences among the various coated implants were not significant. These observations were in correlation with the percentage of bone in contact with the implant surface, as we found a significantly lower percentage of bone in contact with the Ti implants as compared to the Ca-P-coated implants. Despite this difference in connective tissue length, no inflammatory reaction could be seen around any of the implants. Since no perpendicular oriented connective tissue fibres could be demonstrated in any of these sections, we can only conclude that the connective tissue was adapted to the implant surface by fibres which ran parallel or circular to the implant surface. Apparently, such a connective tissue fibre adaptation is sufficient to ensure a biological seal between the contaminated environment of the oral cavity and the almost aseptic internal environment [36].

6.5. CONCLUSION

In summary, although our experiments appear to demonstrate that plasma-sprayed Ca-P coatings deposited on oral implants are beneficial for the trabecular bone reaction, no final conclusion can be drawn due to the difference in surface roughness between the coated and non-coated implants. Consequently, the clinical efficacy of Ca-P plasma-sprayed coated implants can still be questioned. Nevertheless, the observed high loss of machined threaded titanium implants was most surprising and not reported earlier.

REFERENCES

1. **R. Adell**, B. Eriksson, U. Lekholm, P.I. Brånemark, T. Jemt, "A long-term follow-up study of osseointegrated implants in the treatment of fully edentulous jaws", Int J Oral Maxillofac Implant, 5, 3457-3359 (1990).
2. **I. Naert**, M. Quirynen, D. van Steenberghe, "A six year prosthodontic study of 509 consecutively inserted implants for the treatment of partial edentulism", J. Prosth. Dent., 67, 236-245 (1992).
3. **D. van Steenberghe**, B. Klinge, U. Linden U, M. Quirynen, I. Hermann, C.H. Garpland, "Periodontal indices around natural and titanium abutments", J. Periodontol., 64, 538-541 (1993).
4. **T. Jemt**, "Fixed implant supported prostheses in the edentulous maxilla", Clin. Oral Impl. Res., 5, 142-147 (1994).
5. **T. Albrektsson**, L. Sennerby, "State of the art in oral implants", J Clin Periodontol ,18, 474-481 (1991).
6. **U. Lekholm** and G.A. Zarb, "Patient selection and preparation", In P.I. Brånemark, G. Zarb, T. Albrektsson: "Tissue integrated prosthesis: Osseointegration in clinical dentistry." Chicago; Quintessence Publ. Co. Inc. (1985).
7. **K. de Groot**, "Degradable ceramics", In: Biocompatibility of Clinical Implant Materials, vol.1 (ed: D.F. Williams), CRC Press, Inc., Boca Raton: 199-222 (1981).
8. **H. Aoki**, "Science and Medical Applications of Hydroxyapatite", JAAS, Takayama Press System Center co., inc. Tokio (1991).
9. **M. Jarcho**, J.F.Kay, K.I. Gumaer, R.H. Doremus, H.P. Drobeck, "Tissue, cellular and subcellular events at a bone-ceramic hydroxyapatite interface", J. Bioeng., 1, 79-92 (1977).
10. **M. Jarcho**, "Calcium-phosphate ceramics as hard tissue prosthetics", Clin. Orthopaed. and Related Res., 157, 259-278 (1981).
11. **W.J.A. Dhert**, C.P.A.T. Klein, J.A. Jansen, E.A. van der Velde, R.C. Vriesde, P.M. Rozing, and K. de Groot, "A histological and histomorphometrical evaluation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 27, 127-138 (1993).
12. **J.A. Jansen**, J.P.C.M. van der Waerden, K. de Groot, "Development of a new percutaneous access device for implantation in soft tissues", J. Biomed. Mater. Res., 25, 1535-1545 (1991).
13. **M.T. Manley**, "Calcium-phosphate biomaterials: a review of the literature", In: Hydroxylapatite Coatings in Orthopaedic Surgery, (eds. R.G.T. Geesink and M.T. Manley), Raven Press, New York, 1-24 (1993).
14. **C. de Putter**, "Permucosal dental implants of dense hydroxylapatite", Phd thesis, Free University, Amsterdam (1984).
15. **K. de Groot**, R. Geesink, C.P.A.T. Klein, P. Serekian, "Plasma sprayed coatings of hydroxylapatite", J. Biomed. Mater. Res., 21, 1375-1381 (1987).
16. **J.F. Kay**, "Calcium-phosphate coatings for dental implants", Dent. Clin. of North Am., 36, 1-18 (1992).
17. **M. Weinlaender**, E.B. Kennedy, V. Lekovic, J. Beumer, P.K. Moy, S. Lewis, "Histomorphometry of bone apposition around three types of endosseous dental implants", Int. J. Oral Maxillofac. Impl., 7, 491-496 (1992).
18. **J.G.C. Wolke**, J.M.A. de Blieck-Hogervorst, W.J.A. Dhert, C.P.A.T. Klein

CPAT, K. de Groot, "Studies on the thermal spraying of apatite bioceramics", J. of Thermal Spray Technol., 1, 75-82 (1992).

19. **C.A. van Blitterswijk**, Y.P. Bovell, J.S. Flach, H. Leenders, I. van den Brink, J. de Bruijn, "Variations in hydroxylapatite crystallinity: effect on interface reactions", In: Hydroxylapatite Coatings in Orthopaedic Surgery, (eds. R.G.T. Geesink, M.T. Manley), Raven press, Ltd., New York, 33-47 (1993).
20. **W.J.A. Dhert**, C.P.A.T. Klein, J.G.C. Wolke, E.A. van der Velde, K. de Groot, P.M. Rozing, "A mechanical investigation of fluorapatite, magnesiumwhitlockite and hydroxyapatite plasma-spray coatings in goats", J. Biomed. Mater. Res., 25, 1183-1200 (1991).
21. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, "Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbits", J. Biomed. Mater. Res., 27, 603-610 (1993).
22. **B. Chehroudi**, J. Ratkay, D. Brunette, "The role of implant surface geometry on mineralization *in vivo* and *in vitro*; a transmission and scanning electron microscopy study", Cells Mater., 2, 89-104 (1992).
23. **R.M. Pilliar**, D.A. Deporter, P.A. Watson, M. Pharoah, M. Chipman, N. Valquette, S. Carter, K. de Groot, "The effect of partial coating with hydroxyapatite on bone remodeling in relation to porous-coated titanium-alloy dental implants in the dog", J. Dent. Res., 70 (10), 1338-1345 (1991).
24. **D. van Steenberghe**, "The applicability of osseointegrated oral implants in the rehabilitation of partial edentulism: a prospective multicenter study on 558 implants", Int. J. Oral Maxillofac. Impl., 5, 272-281 (1990).
25. **I. Naert**, "The influence of the prosthetic design and the implant type on the tissue reactions around oral implants", Phd thesis. Leuven: Leuven University Press (1991).
26. **H. Caulier**, J.P.C.M. van der Waerden, Y.C.G.J. Paquay, J.G.C. Wolke, W. Kalk, I. Naert, and J.A. Jansen, "The effect of Calcium-phosphate (Ca-P) coatings on trabecular bone response; a histological and clinical study", J Biomed Mater Res, 29, 1061-1069 (1995).
27. **H.B.M. van der Lubbe**, C.P.A.T. Klein, K. de Groot, "A simple method for preparing thin (10 µm) histological sections of undecalcified plastic embedded bone with implants", Stain Technol., 63, 171-177 (1988).
28. **C.P.A.T. Klein**, Y.M.H.F. Sauren, W.E. Modderman, J.P.C.M. van der Waerden, "A new saw technique improves preparation of bone sections for light and electron microscopy". J.Applied Biomater., 5, 369-373 (1994).
29. **K. Søballe**, "Hydroxyapatite ceramic coating for bone implant fixation. Mechanical and histological studies in dogs", Acta Orthopaed. Scand., Supplementum no. 255, 64 (1993).
30. **R.G. Courteney**, M.V. Kayser, S. Downes, "Comparison of the early production of extracellular matrix on dense hydroxyapatite and hydroxyapatite-coated titanium in cell and organ culture", Biomater., 16, 489-495 (1995).
31. **I.M.O. Kangasniemi**, C.C.P.M. Verheyen, E.A. van der Velde, K. de Groot, "*In vivo* tensile testing of fluorapatite and hydroxylapatite plasma-sprayed coatings", J. Biomed. Mater. Res., 28, 563-572 (1994).
32. **K. Gotfredsen**, L. Nimb, E. Hjörting-Hansen, J.S. Jensen, A. Holmen, "Histomorphometric and removal torque analysis for TiO2-blasted titanium implants", Clin. Oral Impl. Res., 3, 77-84 (1992).
33. **J.A. Jansen**, J.P.C.M. van der Waerden, K. de Groot, "Epithelial reaction to percutaneous implant materials: *in vitro* and *in vivo* experiments", J. Invest.

Surg., 2, 29-49 (1989).
34. **J.A. Jansen**, J.P.C.M. van der Waerden, H.B.M. van der Lubbe, and K. de Groot, "Tissue response to percutaneous implants in rabbits", J. Biomed. Mater. Res., 24, 295-307 (1990).
35. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, and K. de Groot, "Histological evaluation of the osseous adaptation to titanium and hydroxyapatite-coated titanium implants", J. Biomed. Mater. Res., 25, 973-989 (1991).
36. **M.A. Listgarten**, N.P. Lang, H.E. Schroeder, A. Schroeder, "Periodontal tissues and their counterparts around endosseous implants", Clin. Oral Impl. Res., 2, 1-19 (1991).

Acknowledgements:
The authors acknowledge Nobel Biocare AB, Gothenburg, Sweden for supplying the Mk II implants.

Chapter 7

THE RELATIONSHIP OF SOME HISTOLOGICAL PARAMETERS, RADIOGRAPHIC EVALUATIONS AND PERIOTEST MEASUREMENTS OF ORAL IMPLANTS; AN EXPERIMENTAL ANIMAL STUDY

H. Caulier[1,2], I. Naert[1], W. Kalk[2], J.A. Jansen[2]

[1] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[2] Department Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

7.1. INTRODUCTION

The best way to ensure that an oral implant is anchored by a rigid union of vital bone is to demonstrate a direct bone apposition to the implant surface at the light microscopic and ultrastructural level [1]. Since this is only possible in the case of implants installed for experimental purposes, several diagnostic methods have been developed for clinical evaluation of the implant status [2].
At present only crude clinical parameters are available to evaluate the success of an oral implant. Implant mobility as an indication of failing bone apposition is generally known. The stability of oral implants should be verified at the moment when transmucosal abutments are connected to the endosseous implants. Whereas a clearly visible mobility can always be correlated with an interposition of fibrous tissue, the range from a clinically firm implant to just tangible mobility represents the actual problem zone.
Long-cone radiographs are only of little help in this respect, as the discrimination accuracy of radiographs is limited. The resolution level of an optimal radiographic technique is close to 0.1 mm [3]. Knowing that the size of a fibroblast is at least 10 times smaller, it becomes evident that radiographs cannot be used to exclude the possibility of intervening soft tissues. Therefore, dynamic measurement methods using implant percussion were investigated to assess clinical mobility of oral implants. This has resulted in the development of an electronic device, the Periotest® (Siemens AG, Bensheim, Germany) [4, 5], which has been reported to allow an objective quantitative and reproducible clinical measurement of the stability of bone-to-implant anchorage [6, 10]. The Periotest® device was originally developed to measure dynamically the periodontal reaction after an impact force to the tooth was applied [11]. The Periotest® method is based on the empirical fact that the greater the implant solidity, the higher the deceleration of the tapping rod that touches the implant, and thus the higher the damping effect of the surrounding tissues. This damping effect is not expressed in the measured contact time of the rod, but appears on a digital scale with values ranging from -8 to +50, called the

Periotest® value. The reproducibility of the device is ± 1 Periotest® unit [12].
Besides lack of implant mobility, the second clinical parameter is marginal bone height stability over time. This can be rated by means of long-cone radiographs. However, irradiation hygiene and the fact that radiographs only reveal the marginal bone level mesio-distally, encourage many clinicians to look for other methods. Therefore the most plausible tool for monitoring changes in the attachment level seems to be the periodontal probe [13, 14].
The functional stability inherent in the healed endosseous implant is remarkably different from that of the natural tooth. Detailed *in vivo* information comparing and contrasting the functional tooth and implant support is scarcely available [15]. Consequently, functional parameters that have prognostic value for tooth support cannot be assumed to have value for implants without experimental documentation.
The present study aims to analyse the correlation between the histological and radiological bone level around oral implants installed in the maxilla of goats. In addition, Periotest® values were correlated to the actual bone contact around implants of the same design but with differing surface layers.

7.2. MATERIALS AND METHODS

7.2.1. Experimental design

Sixteen female adult Saane goats with an average weight between 50-80 kg and an age of 24 to 28 months were used primarily used to investigate the bone reaction around different Ca-P-coated screw-shaped implants [16, see chapter 6].
The first and second premolars of the maxillary dentition were bilaterally extracted under general anaesthesia and the alveoli allowed to heal for a period of 4 months. Special care was taken to avoid both root and bone fragment fractures.
Following completion of the bone healing at the extraction sites, 4

commercially pure titanium screw-designed implants with a diameter of 3.75 mm and a length of 10 mm (MK II type, Nobelpharma AB, Gothenburg, Sweden) were installed under general anaesthesia in the edentulous left and right premolar regions. Three of the four implants were coated with a calcium-phosphate plasma-spray coating (fluorapatite, hydroxylapatite, and heat-treated hydroxylapatite) [16 (see chapter 6), 17]. The implants were inserted with the Nobelpharma drilling equipment using a very gentle surgical technique. A total of 64 implants was inserted, with a distance of 7 mm between the implants, according to a balanced split plot design. The implants were covered with a cover-screw of pure titanium and the mucoperiostal flaps were closed with Vicryl® 2-0 sutures. To avoid postsurgical infection, antibiotic (Albipen®) coverage was applied for three days. The implants were left endosseous for six months to permit healing of the alveolar bone.

At the end of this period the animals were subjected to a second operation to perform the abutment connection (length 4 mm, ∅ 4.5 mm). After installation, the abutments were covered with a plastic healing cap. The animals were killed four months later by an overdose of Nembutal® [16, see chapter 6].

7.2.2. Clinical evaluation

The damping characteristics of the implants were determined using the Periotest® (Siemens AG, Bensheim, Germany) device. After sacrificing, the whole maxilla was harvested and the Periotest® values determined immediately, before fixation of the tissue specimens. The jaws were positioned so that the implants were perpendicular to the floor and the tapping handpiece of the Periotest® was held in a horizontal position at a distance of about 2 mm from the abutment surface. The percussion by the Periotest® rod occurred just below the edge of the coronal platform of each abutment. The same Periotest® device was used during all measurements, which was calibrated before starting any of the measurements. All registrations were performed by the same investigator and accomplished within 30 minutes of killing the animals. The mean Periotest® value of two measurements for each implant was

calculated. Data were accepted only when two consecutive values were obtained that differed by no more than one Periotest® unit.

Radiographic examination was performed using a long-cone radiographic technique. To improve the standardisation of the procedure, block biopsies of the left and right half of the maxilla, including the two implants and the adjacent tooth, with the surrounding soft and hard tissues were obtained. The block biopsies were aligned perpendicular to the cone of the apparatus and one radiograph per biopsy was made. Agfa Dentus radiographic films (size 2), all from the same batch, were exposed using a General Electric® x-ray machine with exposure factors of 65 kV, 15 mA, and focal-film distance of 36 cm. All films were developed with a Dürr-Periomat® (Dürr Dental-D-7120 Bietigheim-Bissingen/Germany) automatic dental film processor. On the radiographs, two reference points were identified mesial and distal of the implants: the bone crest (BC) and the top of the abutment (AB) (see Figure 7.1). The distance BC-AB was measured mesial and distal of the implant and the mean calculated. All measurements were done by the same examiner using a vernier calipers (Mitutoyo Digimatic® 500) with a scale up to 0.01 mm. Finally, a correction factor was applied to compensate for possible small errors between the radiological and real length of the implants.

7.2.3. Histological procedure

Following the clinical evaluation, each implant was resected with the surrounding tissues out of the block biopsies and fixed in 10 % buffered formalin solution. After dehydration by alcohol series, the implant tissue specimens were embedded in methylmetacrylate. The implant and tissue block were mounted in a modified inner circular saw microtome [18] and serial sections were prepared, 10 µm in thickness. The sections were made in a buccopalatal direction parallel to the long axis of the implant surface. The sections were stained with methylene blue and basic fuchsin for histomorphometrical evaluation.

7.2.4. Histomorphometrical evaluation

The complete series (from section 1 to 11) of histological sections per implant were gathered from ***five*** randomly chosen implants. Using a light microscope, the first screw thread starting from the top of the implant showing bone contact was rated from the first (number 1) to the last (number 11) section. In these sections it was possible to determine the first thread showing bone contact at the mesial as well as distal side of the implant. In addition, by evaluating all the histological sections of these five implants, the course of the bone level could be defined (Figure 7.2).

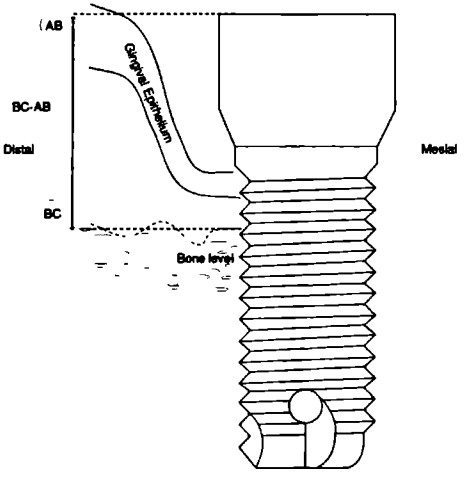


Figure 7.1: Radiographic examination of the implants by measuring the distance BC - AB. (BC = bone crest, AB = top of the abutment)

Subsequently, of the bucco-palatal sections of all implants, the midsection of each implant was used to rate the bone level, defined as the distance from the top of the abutment to the bone crest in contact with the implant surface. Also the number of screw threads and the first screw thread in contact with bone were measured in these midsections. To rate the first screw thread with bone

contact, we numbered the threads in ascending order from the most apical (1) towards the most coronal screw thread (12), see Figure 7.2.

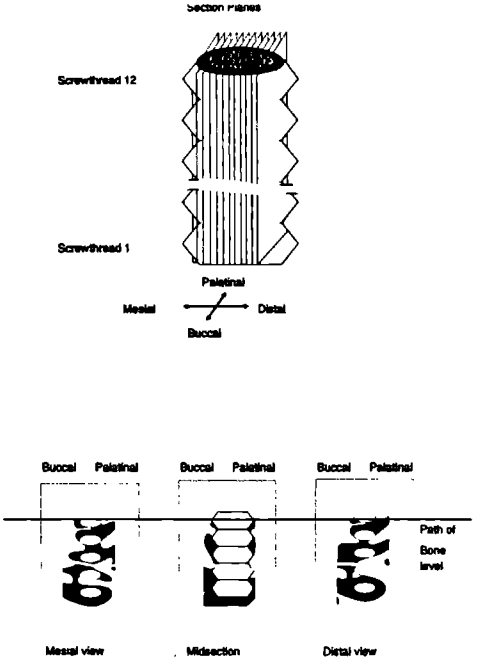


Figure 7.2: The histological sections of the implants were made in bucco-palatal direction, parallel to the long axis of the implants. The first and last section give a mesial and a distal view of the implant. The following histomorphometrical measurements were performed on the histological sections: 1) the first screw thread and 2) the amount of screw threads with bone contact. The screw threads were numbered from 1 (most apical thread) to 12 (most coronal thread). 3) From five randomly chosen implants, the course of the bone level was evaluated in these sections (n = 11).

7.3. RESULTS

During abutment installation it was observed that 10 of the 64 installed

implants were lost or were too mobile to connect with the abutment and were removed. Further, another 6 implants failed during the four months of the permucosal phase. Finally, 10 months after installation, 48 implants could be used for the further examination [16, see chapter 6].

Of the five randomly chosen implants the bone height observed in the eleven histological sections per implant was rated at the same level. Thus there was no difference in the first thread showing bone contact at the mesial or distal and buccal or palatal sides of the implant.

Although a standardised radiological technique was used, only 30 of the 48 radiographs were useful for the radiological examination. These same 30 implants used for the radiological measurements were used for the histomorphometry. The individual and mean values of the radiological and histomorphometrical scores of the bone level are given in Figure 7.3. On average, the histomorphometrical rated bone level was located 0.85 mm more apically compared to the radiological measured bone level. Statistical testing of these data, using a one-way analysis of variance (ANOVA) and a paired t-test revealed that this difference was significant ($p = 0.001$).

Table 7.1 shows the mean Periotest® scores and histomorphometrical data for the different implant materials. A paired t-test only revealed a significantly higher Periotest® value for the titanium implants compared to the Ca-P-coated implants ($p = 0.001$). A simple linear regression analysis was performed to determine if there was a correlation between (1) the observed Periotest® values and the first screw thread showing bone contact, and (2) the Periotest® values and the total number of screw threads showing bone contact for the different implant surfaces. The correlation coefficient (r) revealed that there was neither a correlation ($p > 0.2$) between the Periotest® value and the first screw thread with bone contact for the coated implants ($r = -0.11$) or the non-coated implants ($r = 0.38$), nor between the Periotest® value and the total number of screw threads in contact with bone for the coated implants ($r = -0.17$) or the non-coated implants ($r = 0.42$).

Table 7.1: Mean Periotest® values and histomorphometrical data with standard deviations of the various implant materials; Ti, HA, HAHT, FA

Material	Periotest® Value with SD	Amount# of Screw threads with Bone Contact	First* Screw thread with Bone Contact
Ti	10.1 ± 5.4	5.25 ± 4.9	3.25 ± 3.2
HA	7.4 ± 7.3	11.87 ± 7	7.22 ± 3.8
HAHT	4.3 ± 4.9	11.56 ± 6.1	7.53 ± 3.1
FA	2.9 ± 3.3	13.06 ± 7.2	8.37 ± 3.8

maximum amount of screw threads with bone contact = 12 threads x 2
* the first screw thread with bone contact is calculated from the apex of the implant to screw thread 12 at the top of the implant

7.4. DISCUSSION

There are two reasons that account for the fact that only 30 implants out of the installed 64 implants could be used for the radiographic evaluation. First, the animal model was primarily selected to study the influence of different implant materials on bone behaviour in very spongy bone, such conditions being found in the maxilla of goats. During the months after installation, 48 implants were maintained for further investigation. The results of this part are reported in another paper [16, see chapter 6] and showed the positive effect of Ca-P coatings on implant success during the healing and remodelling period. Second, another 18 implants were excluded from further radiographic investigation because the readability of these radiographs hampered the exact interpretation of the marginal bone height. This was mainly due to the absence of parallelism of both implants per block biopsy. In this context, it has to be noted that only one radiograph per side (left/right) was made.

From a previous study [16, see chapter 6], student paired t-tests revealed no significant difference between the palatal and buccal data for the various parameters for the histomorphometrical evaluations, also used in this study. Therefore, all the histomorphometrical data presented in Figure 7.2 and Figure 7.3 were grouped for analysis.

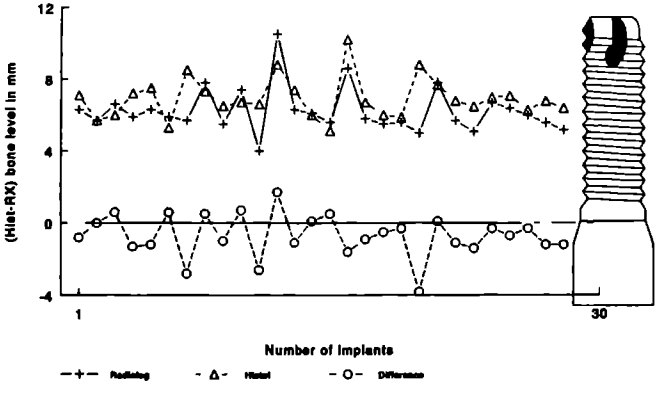


Figure 7.3: This figure represents the mean radiological and histological scores ((buccal + palatal)/2) of the bone level (x-axis) and differences (histological - radiological bone level) of 30 individual implants with optimal radiographs. The values above the bold line represent the values where the RX-values underscored the histological values, those under the line represent the overrated values.

The most commonly used approach to diagnose the bone behaviour around oral implants is radiographic bone examination [19, 20, see chapter 8]. For example, radiography is used to determine the occurrence of vertical bone loss. On the other hand, it is also known that radiography is hampered by some technical difficulties: first, the resolution level is limited, and second, it provides a 2-dimensional representation of a 3-dimensional structure. Therefore, to get more insight into the clinical relevance of radiographs, in the present study we compared the radiological measured bone height around experimental Brånemark® implants with the histological marginal bone height. The measurements revealed that there was a statistical difference between the radiological and histological rated bone level. On average, the radiological data overrated the real marginal bone height by 0.85 mm. Confirmation of this observation was found in a recent study [20, see chapter 8], where the

radiological and histological bone height measurements were performed on the same type of implants inserted in the same implantation region of goats, but in unloaded conditions. On the other hand, the reliability of radiological bone height measurements is, in an individual radiograph, of the order of at least ± 0.5 mm [21]. From these observations, clinicians should be aware that even optimal long-cone radiography mostly leads to a too optimistic image of the reality, and that the standard radiographic technique used in the clinic only gives information in the mesio-distal plane. From this observation it is also shown that the course of bone level around implants in this experiment was equally mesio-distal and bucco-palatal in non-pathological conditions. This strengthens the value of radiographic technique for extrapolating the mesio-distal data to the bucco-palatal. However, we should stress that in this animal study dehiscences or fenestrations never occurred due to favourable alveolar width / implant diameter ratio. From clinical observations it is known that these conditions are not always met in patients.

Besides bone height measurements, the lack of stability of implants is an important parameter for monitoring implant success as well. The latter was indirectly investigated by the clinical efficacy and predictability of the Periotest® device. Periotest® values measured on the retrieved implant-bone specimens were related to the histological bone-to-implant contact area. No correlation could be found. Considering these findings, interpretation of the Periotest® data for evaluation of osseointegration has to be clarified. The Periotest® was originally designed to measure the damping properties of the periodontal ligament around natural teeth. The clinical functioning of implants is based on the achievement of direct bone apposition. However, it has to be noted that the implant-bone interface will always consist of a mixture of fibrous and bone tissue. We assume that the Periotest® data only reflect the mechanical properties of this fibre-osseous complex, as the force of the rod of the Periotest® transmitted to the implant can be expressed as an impulse. This impulse is transmitted to the bone. The deceleration of the rod is thus dependent on the damping capacities and Young's modules of the implant-

surrounding tissues [6, 10]. The Periotest® values found in this study were neither correlated with the bone level in relation to the implant body, nor with the amount of threads in contact with bone. The Periotest® should provide the clinician with information about the implant support that can be combined with other appropriate clinical data to arrive at a diagnosis. Lower Periotest® values are assumed to be indicative of favourable and predictive implant-bone conditions. Assuming this to be true, the relationship of Periotest® values to bone contact would be expected to be inverse and strong. However, this relationship was not found to support this hypothesis. Although the mean values of the Periotest® and bone data suggest a correlation, statistical testing did not confirm this finding. To illustrate this, examples from three different HAHT-coated implants with Periotest® values of +2, 0, +4 exhibited a total number of screws in contact with bone of 7, 9, and 8 respectively. While another three HAHT-coated implants with more or less the same Periotest® values of +1, +2, and +3 exhibited double total numbers of screws in contact with bone of 18, 18, 19 respectively. Therefore, the sensitivity and the specificity of the diagnostic device for these observations are poor. A low Periotest® value will thus not exclude an implant anchored with little bone. This means that the reliability of one single Periotest® examination is quite substantial, but perhaps suitable in a consecutive series of measurements of a great number of implants as demonstrated in previous investigations [10, 13]. As the Periotest® device is not able to rate the amount of bone contact with the implant, radiographs or probing of the pocket depth are necessary to follow the marginal bone or attachment level over time.

Finally our results are in contrast with the findings of Carr *et al.* [22] who reported that for unloaded implants, assessed 3 or 4 months post-placement, the Periotest® values are not different for the various implant materials used (c.p. Ti, Ti-alloy, HA). However, in our study the implants were loaded for 4 months by the abutment connection. This discrepancy in observations may be attributed to the loading situation where biomaterial-specific interactions become evident.

7.5. CONCLUSION

This study revealed that radiological data overrated the real marginal bone level around screw-shaped oral implants, and that the Periotest® device is neither able to discriminate between the first thread, nor between the total threads in contact with bone. Studies that investigate the diagnostic value of evaluation techniques, based on an understanding of their sensitivity and specificity compared to established clinical parameters and on a standardisation of histology data addressing the biological support for oral implants, are recommended.

REFERENCES

1. **T. Albrektsson**, B. Albrektsson, "Osseointegration of bone implants: A review of an alternative mode of fixation", Acta Orthopaed. Scand., 58, 567 (1987).
2. **T. Albrektsson**, L. Sennerby, "Direct bone anchorage of oral implants: Clinical and experimental considerations of the concept of osseointegration", Int. J. Prosth., 3, 30-41 (1990).
3. **T. Albrektsson**, M. Jacobsson, "Bone-metal interface in osseointegration", J. Prosth. Dent., 57, 597-607 (1987).
4. **W. Schulte**, "Messung des Dämpfungsverhaltens enossaler Implantate mit dem Periotestverfahren", Deutsche Zeitschrift Zahnärtzliche Impl., 2, 11 (1986).
5. **W. Schulte**, B. d'Hoedt, D. Lukas, M. Maunz, M. Steppeler, "Periotest for measuring periodontal characteristics - Correlation with periodontal loss", J. of Periodontal Res., 27, 184 (1992).
6. **J. Olivé**, C. Aparicio, "The periotest method as a measure of osseointegrated oral implant stability", Int. J. of Oral and Maxillofac. Implants, 5, 390-400 (1990).
7. **J. Teerlinck**, M. Quirynen, P. Darius, D. van Steenberghe, "Periotest®: an objective clinical diagnosis of bone apposition toward implants", The Int. J. of Oral and Maxillofac. Impl. 6: 55-61 (1991).
8. **D.E. Van Scotter**, C.J. Wilson, "The Periotest method for determining implant success", J. of Oral Impl., 17, 410-413 (1991).
9. **M. Manz**, H.F. Morris, O. Shigeru, "An evaluation of the Periotest® system. Part I: examiner reliability and repeatability of readings", Implant Dent., 1, 142-146 (1992).
10. **D. van Steenberghe**, J. Tricio, I. Naert, M. Nys, "Damping characteristics of bone-to-implant interfaces. A clinical study with the Periotest® device", Clin. Oral Impl. Res.,6, 31-39 (1995)
11. **W. Schulte**, B. d'Hoedt, D. Lukas, L. Muhlbrandt, F. Scholz, J. Bretschi,

"Periotest® - a new measuring method for periodontal function", Zahnartzliche Mitteilungen, 11, 2-8 (1983).

12. **B. d'Hoedt**, W.A. Schulte, "A comparative study of results with various endosseous implant systems", Int. J. Oral Maxillofac. Impl., 4, 95-105 (1989).
13. **D. van Steenberghe**, M. Quirynen, "Reproducibility and detection threshold of peri-implant diagnostics", Adv. Dent. Res., 7 (2), 191-195 (1993).
14. **T. Berglundh**, J. Lindhe, I. Eriksson, C.P. Marinello, B. Liljenberg, P. Thomsen, "The soft tissue barrier at implants and teeth", Clin. Oral Impl. Res., 2, 81-90 (1992).
15. **D. Kohn**, "Overview of factors important in implant design", J. Oral Impl., 18, 204-213 (1992).
16. **H. Caulier**, J.P.C.M. van der Waerden, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "A histological and histomorphometrical evaluation of the application of screw-designed calcium-phosphate (Ca-P) coated implants in the cancellous bone of the goat", submitted (1995).
17. **K. de Groot**, R. Geesink, C.P.A.T. Klein, P. Serekian, "Plasma sprayed coatings of hydroxylapatite", J. of Biomed. Mater. Res., 21, 1375-1381 (1987).
18. **C.P.A.T. Klein**, Y.M.H.F. Sauren, W.E. Modderman, J.P.C.M. van der Waerden, "A new saw technique improves preparation of bone sections for light and electron microscopy", J. of Applied Biomaterials, 5, 369-373 (1994).
19. **T. Albrektsson**, G. Zarb, P. Worthington & A.R. Eriksson, "The long-term efficacy of currently used dental implants, a review and proposed criteria of success", Int. Journal of Oral and Maxillofac. Impl., 1, 11-25 (1986).
20. **H. Caulier**, I. Naert, W. Kalk, J.P.C.M. van der Waerden, S. Vercaigne, J.A. Jansen, "The effect of Ca-P plasma-sprayed coatings on the initial bone healing of oral implants; an experimental study in the goat", accepted in J. Biomed. Mater. Res.(1996).
21. **K. Gröndahl**, B. Kullendorff, K.G. Stridh, H.G. Gröndahl, C.O. Henrikson, "Detectability of artificial marginal bone lesions as a function of lesion depth. A comparison between substraction radiography and conventional radiographic technique", J. Clin. Periodontol., 15, 156-162 (1988).
22. **A.B. Carr**, E. Papzoglou, P. Larsen, "The relationship of Periotest values, biomaterial, and torque to failure in adult baboons", Int. J. Prosthodont., 8, 15-20 (1995).

Chapter 8

THE EFFECT OF CA-P PLASMA-SPRAYED COATINGS ON THE INITIAL BONE HEALING OF ORAL IMPLANTS; AN EXPERIMENTAL STUDY IN THE GOAT

H. Caulier[1, 2], S. Vercaigne[1, 2], I. Naert[1], J.P.C.M. van der Waerden[2], J.G.C. Wolke[2, 3], W. Kalk[2], J.A. Jansen[2]

[1] Department of Prosthetic Dentistry, BIOMAT research group, School of Dentistry, Oral Pathology and Maxillofacial Surgery, University of Leuven, Belgium

[2] Department Oral Function, Laboratory of Biomaterials, Dental School, University of Nijmegen, The Netherlands

[3] Department of Biomaterials, University of Leiden, The Netherlands

8.1. INTRODUCTION

The use of oral implants has grown expansively during the last 15 years because of the increased demand for dental care of patients who have lost their teeth due to trauma, disease or neglect. This resulted in the development of numerous oral implant systems. Currently, with several of the available systems, clinical success rates of 90-98 % [1-4] are reached over more than 3 year follow-up periods when the implants are installed in the symphyseal area of the mandible. Poorer results are found when implants are inserted in bone of lower density as found in the maxilla and posterior regions of the mandible.
In addition to location, many other factors can influence the success or failure of an oral implant. Of primary importance is the used surgical technique. Surgery has to be performed in an atraumatic way, for example by the use of adequate cooling during surgery and low rotational drill speed (max. 450 rpm) [5, 6]. Too much surgically destroyed tissue will have a negative effect on the chances for proper tissue repair.
Secondary factors that can delay the healing of implants are of biomechanical origin. Early movement of the implant before the surrounding bone is laid down to the metal surface and overload during the initial healing phase [7-9] will lead to a predominant formation of interfacial connective tissue. Therefore, implants are usually not loaded earlier than after a healing period of 3 months when they are placed in bone of high mineral density as found in the lower jaw. In bone of low density as found in the upper jaw, at least 6 months is waited before loading, as it is supposed that wound healing and bone repair will take longer in the latter type of bone.
In light of the above mentioned, we investigated in a previous study [10, see chapter 6] whether calcium-phosphate (Ca-P) coatings can improve the bone response to oral implants placed in low density bone of the maxilla of goats. Histomorphometrical evaluation of the retrieved implants revealed a significantly greater percentage of direct trabecular bone contact to the Ca-P-coated implants compared to non-coated implants. In this previous study the

same surgical protocol was used as for the treatment of patients. The implants were left endosseous for six months to allow healing of the alveolar bone before the permucosal abutments were connected. In addition to the overall bone response another suggested advantage [11-13] of Ca-P coatings is the improvement of the initial bone response. If the latter hypothesis is indeed true, waiting for six months is superfluous and the intervening healing period could be reduced.

Therefore, the aim of this study was to determine the effect of Ca-P coatings on the bone response to oral implants inserted into maxillary bone of low density of goats 3 and 6 months after installation.

8.2. MATERIALS AND METHODS

8.2.1. Implant materials and coating characteristics

Forty eight threaded commercially pure titanium implants with a length of 10 mm were manufactured. Twelve of the implants were left non-coated and had a diameter of 3.75 mm. The other implants had a diameter of 3.65 mm and were subjected to a plasma-spray coating technique with Ca-P. Before coating, these implants were grit-blasted with Al_2O_3 (Ra = 4-5 µm), cleaned ultrasonically in propanol and dried at 100°C. Three different coatings were deposited:

- Hydroxyapatite (HA)
- Hydroxyapatite subjected to a heat-treatment (650 °C during 10 minutes) (HAHT)
- Fluorapatite (FA)

The prepared coatings had a thickness of about 50 µm and were characterized by X-ray diffraction (XRD) and infra-red spectroscopy (IR) [14, see chapter 3]. In summary this analysis revealed that the HA coatings had a crystallinity of 60 %, the HAHT-coatings of 65 %, and the FA-coatings of 95 %. The final diameter of all implants, coated and non-coated, was 3.75 mm.

Before surgery, all implants were cleaned ultrasonically in 100 % ethanol and dried at 50 °C. The implants were sterilized in a steam autoclave.

8.2.2. Experimental design and surgical procedure.

Twelve healthy adult female Saane goats, with an average age of 30 months and an average weight of 65 kg were selected for the experimental animal model. The animals were kept in quarantine for at least 4 weeks, and tested for CAE/CL arthritis.

For the insertion of the implants the two first maxillary premolars were bilaterally extracted. The extraction was performed under general anesthesia induced by intravenous pentobarbital 25 mg/kg and atropine 0.5 mg/animal. After oro-tracheal intubation, anesthesia was performed by ethrane through a constant volume ventilator. The extraction wounds were closed with resorbable Vicryl® 2-0 sutures.

After a healing period of four months, the goats were again subjected to a general anesthesia for the installation of the implants. The operation field was washed with 0.1 % chlorhexidine. A longitudinal incision was made on the palatal site of the edentulous region of the alveolar ridge and a mucoperiosteal flap was raised. After exposition of the bone, two holes were made with a guide drill. The distance between the two holes was 7 mm. Subsequently, the implant sites were further prepared to their final depth and width. The diameter of the last burr was 3.5 mm. This allowed a good initial stability of all the implants after installation. The bone preparation was performed with a very gentle surgical technique and the implant placement was done under abundant irrigation with a cold saline solution. Finally, the operation site was cleaned thoroughly by rinsing before closing the mucoperiosteal flaps with Vicryl® 2-0 sutures.

A total of 48 implants was placed; 12 Ti, 12 coated HA, 12 coated HAHT, and 12 coated FA implants. Each animal received all types of implants, two in the left and two in the right half of the maxilla. The implants were placed according

to a balanced split plot design in order to compensate for differences in bone quality and quantity between the implantation sites.

To reduce the peroperative infection risk, prophylactic antibiotics (Albipen®) were administered for three days starting one hour postoperatively.

The protocol did foresee to kill six of the twelve goats after 3 months and the other six after 6 months by an overdose of pentobarbital (Nembutal®). Following the death of the goats, segments of the maxilla were removed and divided in small blocks containing the implants.

8.2.3. Radiographic examination.

Radiographs were taken of the block biopsies of the maxilla which contained the implants. The cone of the apparatus was oriented perpendicular to the implants. One radiograph was taken of each biopsy, using a General Electric® X-ray machine with exposure factors of 65 kV, 15 mA, and focal-film distance of 36 cm. All films were developed with a Dürr-Periomat® (Dürr Dental-D-7120 Bietigheim-Bissingen/Germany) automatic dental film processor. The distance between the top of the neck (TN) of the implant and the bone crest (BC) was measured for each implant (Figure 8.1) on the mesial and distal site and the mean was calculated. These measurements were performed by the same investigator with a vernier calipers (Mitutoyo Digimatic® 500) with a scale up to 0.01 mm.

8.2.4. Histological procedure.

After radiographic examination the block biopsies were fixed in 10 % buffered formalin solution, dehydrated in alcohol series, embedded in methylmethacrylate and sectioned for light microscopical assessment with a modified inner circular saw microtome [15,16]. The sectioning technique provided 10 µm thick serial sections in bucco-palatal direction parallel to the long-axis of the implant surface. The sections were stained with methylene blue and basic fuchsin.

8.2.5. Histological and histomorphometrical evaluation.

First, a descriptive evaluation of the trabecular bone response to the various implants was performed.

Second, the bone reaction was histomorphometrically assessed using a light microscope connected to a computer equipped with a video and image analysis system (Technical Command Language®-image). For this analysis, three representative sections of each implant were selected.

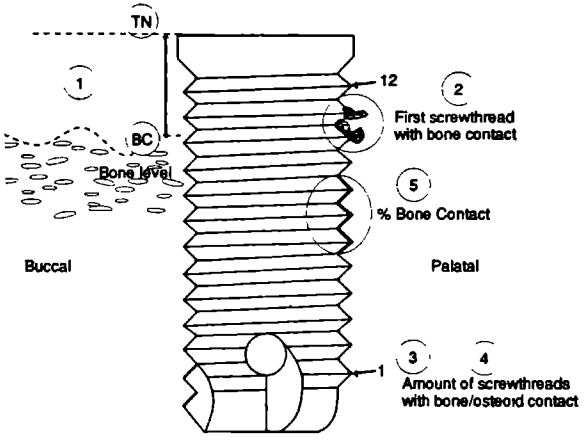


Figure 8.1: Schematic drawing of the parameters used for the histological, histomorphometrical and radiological evaluation:

1. bone level measured as the distance from the top of the neck of the implant (TN) to the bone crest (BC) in contact with the implant surface.
2. first buccal and palatal screw thread showing bone contact
   1 = most apical screw thread
   12 = most coronal screw thread

3, 4. the average of the number of buccal and palatal screw threads with direct bone/osteoid contact

5. percentage of direct bone contact

The following parameters (Figure 8.1) were evaluated:

1. The buccal and palatal marginal bone level, rated as the distance from the top of the neck of the implant to the bone crest in contact with the implant surface.
2. The first buccal and palatal screw thread with direct bone contact by numbering the screw threads in ascending order from the most apical (1) towards the most coronal screw thread (12).

3, 4. The average of the number of buccal and palatal screw threads with direct bone/osteoid contact.

5. The percentage of direct bone contact along the three best consecutive screw threads.

8.3. RESULTS

One goat of the 3-months installation group had to be sacrificed two weeks after implant installation due to a broken leg. As this happened in an early period after insertion of the implants, this animal was excluded from the histological and histomorphometrical evaluation. At the end of the implantation periods, it appeared that of the remaining 44 installed implants 3 implants were lost (not in situ) in the three-months group and 6 in the 6-months group (Table 8.1). A Chi-square test revealed that there was no significant difference in loss or maintenance between the Ca-P-coated and non-coated implants, neither for the 3-months, nor for the 6-months group (respective p-values 0.7 and 0.1).

8.3.1. Histological description.

Examination of the histological sections showed that 7 of the 35 maintained implants were located partially in the maxillary sinus. Apparently, this position did not influence the final tissue response. In addition, we noticed no difference in bone reaction between the 3 and 6 months group. Around all implants abundant fibrous tissue was present at both installation periods

(Figure 8.2). Mostly this fibrous tissue contained a high amount of plasma cells (Figure 8.3).

Table 8.1: Loss and maintenance of the different implant materials for the different implantation periods.

Material	3 months		6 months	
	Maintained	Lost	Maintained	Lost
FA	4	1	5	1
HAHT	4	1	5	1
HA	5	0	5	1
Ti	4	1	3	3
Total	17	3	18	6

FA = fluorapatite
HAHT = hydroxyapatite heat treated
HA = hydroxyapatite
Ti = titanium

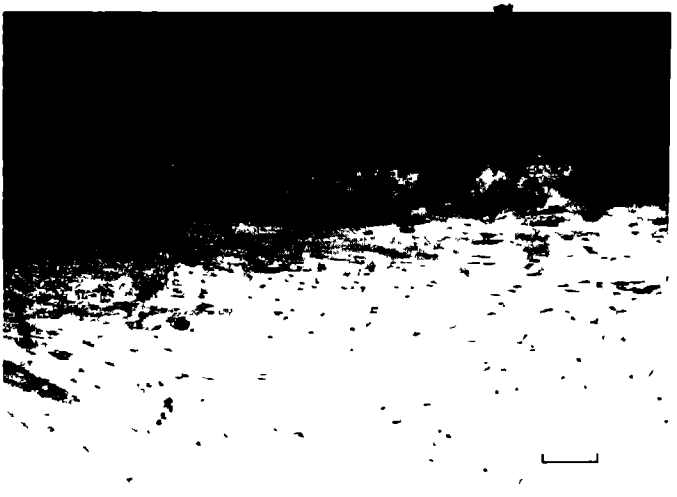


Figure 8.2: Light micrograph showing fibrous tissue formation around a FA-coated implant 3 months after implantation. Despite the presence of fibrous tissue the coating is still intact.
original magnification: 50 x, bar = 50 µm

The Ca-P-coated implants appeared to induce more bone formation than the non-coated implants (Figure 8.4). The newly formed bone was mainly observed into the screw threads. No intervening fibrous tissue layer could be observed between the implant surface and the contacting bone (Figure 8.5). Occasionally, osteoid formation was seen (Figure 8.6). Further, we observed that at some sites bone was closely laid down towards the implant even when the implant was almost completely surrounded by inflammatory tissue. At the coronal cortical level, around all coated and non-coated implants, a gap existed between the bone and implant (Figure 8.7).

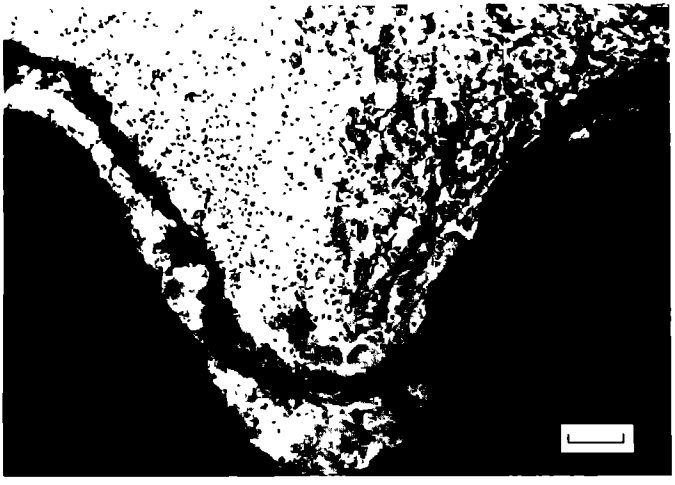


Figure 8.3: Light microscopical section of a FA-coated implant 3 months after implantation. Plasma cells were seen in the connective tissue surrounding the implant. On the coated surface macrophages (arrows) were present. This inflammatory reaction did not result in coating loss.
original magnification: 50 x, bar = 50 µm

Qualitatively, it appeared that all three coatings reduced in thickness after the 3 month as well as after the 6 month implantation period. This reduction was not uniform. At some places the entire coating thickness was maintained, while at other sites only a thin layer or no coating was left. This coating reduction

was more severe for the HA-coatings than for the HAHT-and the FA-coatings. Frequently, cellular activity of multinucleated cells could be observed in the vicinity of the coated implant surfaces (Figure 8.8). Nevertheless, these cells could not be associated with the coating reduction. In addition, no relation could be found between the presence of fibrous tissue, inflammatory cells, or bone tissue and coating degradation (Figure 8.9).

Table 8.2: Percentages of bone contact for the various maintained implants for both implantation periods.

Percentage of Bone Contact along the Three Best Consecutive Screw threads				
	3 months		6 months	
Material	%	Mean with SD	%	Mean with SD
FA	68	27 ± 33	44	26 ± 13
FA	1		20	
FA	0		34	
FA	39		10	
FA			23	
HAHT	0	16 ± 31	40	22 ± 16.4
HAHT	63		0	
HAHT	2		35	
HAHT	0		12	
HAHT			22	
HA	58	36 ± 20	54	35 ± 24
HA	12		63	
HA	49		34	
HA	18		2	
HA	44		23	
TI	30	11 ± 13	0	15 ± 26
TI	6		0	
TI	8		45	
TI	0			

FA = fluorapatite
HAHT = hydroxyapatite heat treated
HA = hydroxyapatite
Ti = titanium

Figure 8.4 Histological appearance of a FA-coated implant 3 months after implantation demonstrating bone deposition on the implant surface No intervening fibrous tissue layer can be observed at the interface
A original magnification 10 x, bar = 294 µm
B original magnification 40 x, bar = 73 µm

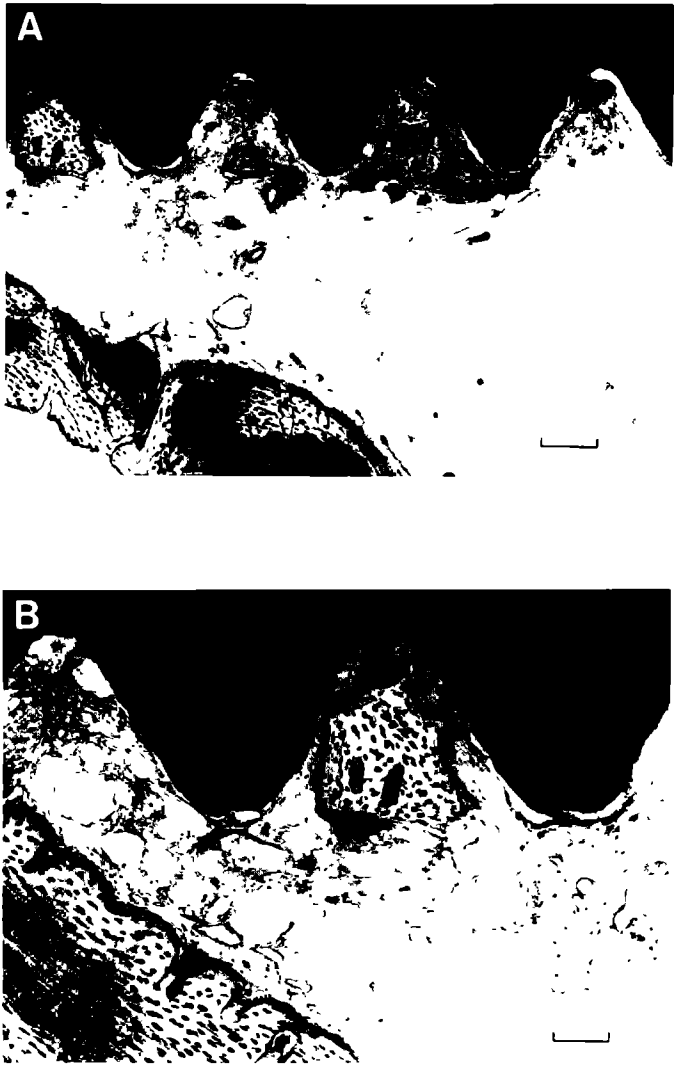


Figure 8.5: Histological appearance of a non-coated titanium implant 6 months after implantation showing limited bone formation into the screw threads.
A original magnification. 12 5 x, bar = 204 µm
B. original magnification: 25 x, bar = 118 µm

8.3.2. Histomorphometrical evaluation.

Tables 8.2, and 8.3 show all results of the histomorphometrical evaluation of the 3 and 6 months specimens.

At 3 months a wide interanimal variation in bone contact percentages for FA, HAHT and Ti implants was observed. For HA implants and after 6 months of implantation, the results were more consistent. Further, the average percentage of bone contact of Ca-P-coated implants appeared to be higher than for non-coated implants. Nevertheless, statistical analysis using a one way analysis of variance and Tukey multiple comparison procedures revealed that this difference was not significant ($p > 0.5$). In addition, no difference existed in amount of bone contact between the 3 and 6 months implants ($p > 0.5$).

Table 8.3: Means and standard deviations of the first screw thread° and number of screw threads with bone contact for the maintained different implant materials for both implantation periods.

	3 months			6 months		
Bone Contact	First Thread		Number of Threads	First Thread		Number of Threads
Material	Buccal	Palatal		Buccal	Palatal	
FA	4 (± 5)	6 (± 6)	5 (± 6)	10 (± 5)	9 (± 3)	4 (± 2)
HAHT	2 (± 4)	3 (± 4)	3 (± 5)	6 (± 5)	6 (± 6)	5 (± 5)
HA	7 (± 5)	11 (± 1)	9 (± 5)	6 (± 5)	9 (± 1)	6 (± 3)
TI	5 (± 6)	5 (± 5)	3 (± 3)	4 (± 7)	4 (± 6)	4 (± 6)

°no 1 = most apical screw thread and no 12 = most coronal screw thread
Standard deviations between parentheses
FA = fluorapatite
HAHT = hydroxyapatite heat treated
HA = hydroxyapatite
Ti = titanium

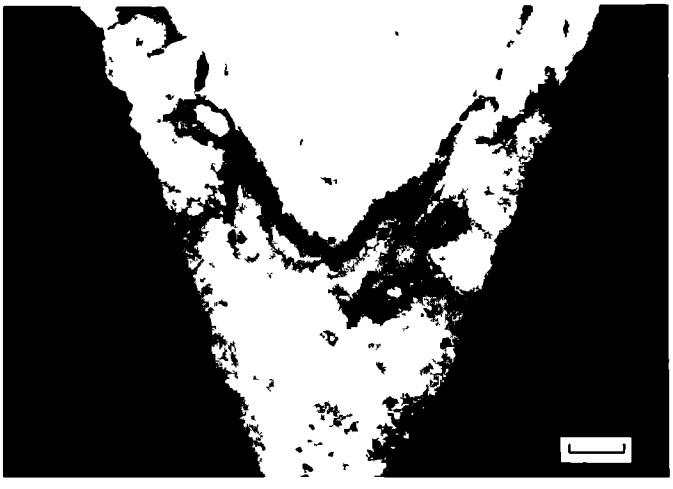


Figure 8.6 Osteoid formation on a FA-coated implant 3 months after insertion was observed Osteoblasts are present on top of the osteoid
original magnification 50 x, bar = 50 µm

Figure 8.7 Bone reaction around an HAHT implant 3 months after insertion A gap was present at the coronal level (original magnification 10 x, bar = 294 µm)

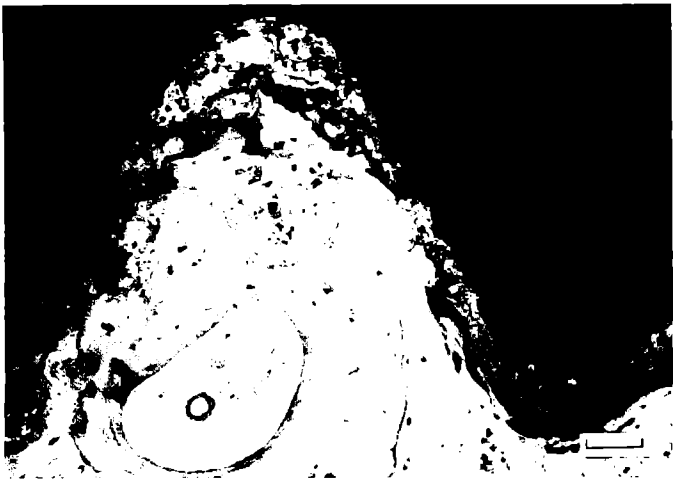


Figure 8.8: Histological section of a FA-coated implant 6 months after insertion. Newly formed bone can be observed into the screw thread. Despite cellular activity in the remodeling lacuna on the implant surface there was no sign of coating degradation. original magnification: 50 x, bar = 50 µm

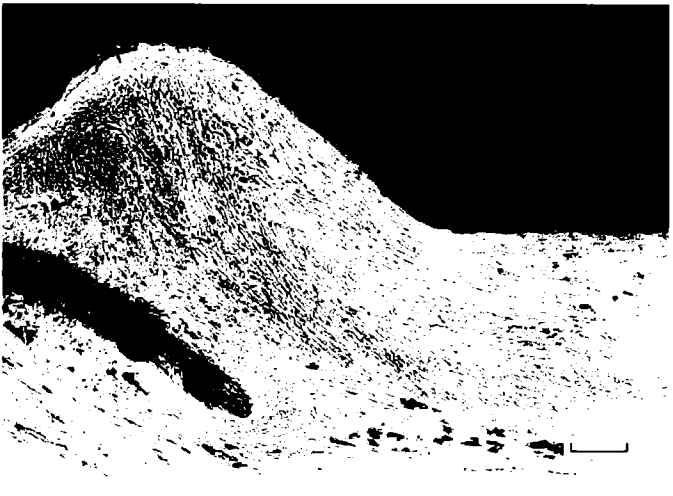


Figure 8.9: Histological appearance of a FA-coated implant 6 months after implantation. The coating has completely disappeared.
original magnification: 25 x, bar = 118 µm

Further examination showed that:

- The first palatal screw thread with bone contact in the 3 months specimens was located more coronally for the HA implants compared to all other implants ($p < 0.05$). On the other hand, buccally only a difference was observed between the HA- and the HAHT implants ($p < 0.05$). At 6 months a significant difference in the first thread with bone contact was observed between FA- and Ti- implants for the buccal side and between HA- and Ti-implants for the palatal side ($p < 0.05$).
- For the number of screw threads with bone contact only a significant difference ($p < 0.05$) existed between HA- and Ti-implants, 3 months after implantation.

Finally, using a Student's t-test, we compared the radiological and histological rated marginal bone level. This test demonstrated that the radiographs overrated significantly ($p < 0.001$) the bone level 3.1 mm more coronally than the histological rated bone level.

8.4. DISCUSSION

In this study less non-coated titanium implants were lost at 3 and 6 months post-implantation compared to our previous studies [10, 17, see chapter 6 and 7] where the same animal model and implant materials were used. This difference can be explained by the increased skill of the surgeon as the maxilla of the goat is rather difficult to access. Further, we observed that the initial stability of the implants after installation in the present study was higher. This can also be a contributory factor to the improved survival percentage. This increased stability was obtained by omitting the countersink drill for the adjustment of the alveolar ridge to the shape of the implant neck, because of the soft bone.

Although the clinical success was satisfying, a much lower percentage of bone contact was found for the various implant materials compared to the previous

studies (Ca-P: 60-79 %; Ti: 26 %) [10, 17, see chapter 6 and 7]. In addition, no significant difference was observed in bone reaction in the present study between the various implant materials. It can be supposed that this is due to the absence of mechanical stimulation of the implants. Indeed the implants in the above mentioned study were connected with a permucosal abutment with a plastic healing cap on top of it which occluded with the mandibular teeth (premolars).

Nevertheless, the results of this experiment are far inferior compared to the results of some clinical investigations. For example, titanium screw-shaped implants have proven to be successful in various clinical circumstances [18, 19]. The same holds true for the Ca-P-coated implants when comparing with other experimental studies [20, 21, see chapter 5]. The inferior bone reaction as found in the present study must be attributed to the used animal model as the implants were installed in an host bed of extremely low mineralisation and density.

Another interesting finding in this study is that the histological and histomorphometrical results revealed no differences in average bone reaction to the various implant surfaces for the 3 and 6 months installation period. This observation is probably due to the observed wide inter and intra-animal variation in bone contact percentages as observed for the FA and HAHT-coated and the non-coated implants. Such a variation was not present for the HA-coated implants. This last result appears to confirm the earlier suggested advantage of less crystalline plasma-sprayed Ca-P coatings [22]. The degradation of the amorphous component causes an increased calcium-phosphate precipitation resulting in a more rapid bone bonding. Therefore, the questions arise whether: a. the use of very amorphous plasma-sprayed HA coatings can reduce further the intervening healing period before loading an oral implant, and b. this effect is lasting, even after complete resorption of the layer and loading of the implant.

Although standard radiography is a valuable clinical tool to monitor marginal bone level changes over time, taking into account the maximal resolution level

of 0.1 mm [23], the histomorphometrical data significantly underscore the radiologically determined marginal bone level. These results are in agreement with earlier observations [17, see chapter 7]. It has also to be reminded that the histomorphometrically determined marginal bone level was rated in the bucco-palatal plane, the radiographs reveal the bone level in the mesio-distal plane. Although in humans real differences may exist due to dehiscences, in the goat due to the rather large bucco-palatal width of the alveolar crest, this hardly occurs [17, see chapter 7].

All coatings showed reduction in thickness as already found in previous studies [14 (see chapter 3), 24, 25]. Nevertheless, the reduction was more severe for the HA-coated implants compared to the FA- or the HAHT- coated implants. However, as already discussed in previous publications [14 (see chapter 3), 25], the reason and consequences of this coating loss are still not understood. The degradation of the coating is not related to the presence of multinucleated or inflammatory cells, fibrous tissue or bone tissue. Probably the reduction of the coating thickness is due to the dissolution of the amorphous phase between the remaining crystalline coating particles. Therefore, supported by this and earlier results [14 (see chapter 3), 24-26], we suppose that the amount of coating reduction is probably determined by the animal model (species) and wound healing capacity of the bone (bone type and implantation site).

8.5. CONCLUSION

In summary, although we are aware of the fact that extrapolation of results of animal studies to the human situation is difficult, the results of this study suggest that it is probably not necessary to wait longer than 3 months before loading maxillary implants when the implants are provided with an amorphous plasma-sprayed HA coating. Further research has to confirm this hypothesis.

REFERENCES

1. **D. Buser**, H.P. Weber, N.P. Lang, "Tissue integration of non-submerged implants: 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants", Clin. Oral Impl. Res., 1, 33-40 (1990).
2. **K. Gotfredsen**, B. Holm, J. Sewerin, F. Harder, E. Hjörting-Hansen, C.S. Pedersen, K. Christensen, "Marginal tissue response adjacent to Astra dental implants supporting overdentures in the mandible. A 2-year follow-up study", Clin. Oral Impl. Res., 4, 83-89 (1993).
3. **M. Quirynen**, I. Naert, D. van Steenberghe, L. Nijs, "A study of 589 consecutive implants supporting complete fixed prostheses. Part I: Periodontal aspects. J. Prosthet. Dent., 68, 655-663 (1992).
4. **D. Buser**, H.P. Weber, U. Brägger, C. Balsiger, "Tissue integration of one-stage ITI-implants: 3-year results of a longitudinal study with hollow-cylinder and hollow-screw implants", Int. J. Oral Maxillofac. Impl., 6, 405-412 (1991).
5. **R. Eriksson**, R. Adell, "Temperatures during drilling for the placement of implants using the osseointegration technique", J. Oral Maxillofac. Surg., 44, 4-7 (1986).
6. **F. Sutter**, "Das ITI-Bonefit-implantsystem-Implantatbettgestaltung", Die Quintessenz, 4, 541-554 (1991).
7. **T. Albrektsson**, P.I. Brånemark, H.A. Hansson, J. Lindström, "Osseointegrated titanium implants", Acta Orthop. Scand., 52, 155-170 (1981).
8. **T. Albrektsson**, B. Albrektsson, "Osseointegration of bone implants: A review of an alternative mode of fixation", Acta Orthopaed. Scand., 58, 567 (1987).
9. **W.E. Roberts**, "Bone tissue interface", J. Dent. Ed., 52(12), 804-809 (1988)
10. H. Caulier, J.P.C.M.van der Waerden, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "A histological and histomorphometrical evaluation of the application of screw-designed Ca-P-coated implants in the cancellous maxillary bone of the goat", J.Biomed. Mater. Res., accepted in J. Biomed. Mater. Res. (1996).
11. **H. Aoki**, "Science and medical applications of hydroxyapatite", Takayama Press System Center co, inc. Tokio (1991).
12. **K. de Groot**, "Degradable ceramics" In: Biocompatibility of clinical implant materials, vol.1 (ed: D.F. Williams), CRC Press, Inc., Boca Raton: 199-222 (1981).
13. **K. de Groot**, J.G.C. Wolke, J.A. Jansen, "State of the art: Hydroxylapatite coatings for dental implants", J.Oral Impl., 20 (3), 232-234 (1994).
14. **H. Caulier**, J.P.C.M. van der Waerden, Y.C.G.J. Paquay, J.G.C. Wolke, W. Kalk, I. Naert, J.A. Jansen, "Effect of Ca-P coatings on trabecular bone response. A histological study", J. Biomed. Mater. Res., 29, 1061-1069 (1995).
15. **C.P.A.T. Klein**, Y.M.H.F. Sauren, W.E. Modderman, J.P.C.M. van der Waerden, "A new saw technique improves preparation of bone sections for light and electron microscopy", J. Appl. Biomater., 5, 369-373 (1994).
16. **H.B.M. van der Lubbe**, C.P.A.T. Klein, K. de Groot, "A simple method for preparing thin (10 µm) histological sections of undecalcified plastic embedded bone with implants", Stain Technol., 63, 171-177 (1988).
17. **H. Caulier**, I. Naert, W. Kalk, J.A. Jansen, "The relationship of some histological parameters, radiographic evaluations and Periotest measurements of oral implants; an experimental animal study", Int. J. Oral Maxillofac. Impl., submitted (1995).

18. **D. van Steenberghe** , U. Lekholm, C. Bolender, T. Folmer, P. Henry, J. Hermann, K. Higuchi, W. Laney, U. Lindérs, P. Astrand, "The applicability of osseointegrated oral implants in the rehabilitation of partial edentulism: a prospective multicenter study on 558 implants", Int. J. Oral Maxillofac. Impl., 5, 272-281 (1990).
19. **I. Naert** , M. Quirynen, D. van Steenberghe, "A six-year prosthodontic study of 509 consecutively inserted implants for the treatment of partial edentulism", J. Prosth. Dent., 67, 236-245 (1992).
20. **K. Søballe**, "Hydroxyapatite ceramic coating for the bone implant fixation.Mechanical and histological studies in dogs", Acta Orthopaed. Scand., supplementum no. 255, 64 (1993).
21. **H. Caulier**, T. Hayakawa, I. Naert, J.P.C.M. van der Waerden, J.G.C. Wolke, J.A. Jansen, "An animal study on the bone behavior of Ca-P-coated implants: influence of implant location", Mater. in Med., submitted (1995).
22. **K. de Groot**, J.G.C. Wolke, J.A. Jansen, "State of the art: Hydroxyapatite coatings for dental implants", J. of Oral Impl., 20, 232-234 (1994).
23. **T. Albrektsson** and L. Sennerby, "Direct bone anchorage of oral implants: Clinical and experimental considerations of the concept of osseointegration", Int. J. Prosthodont., 3, 30-41 (1990).
24. **W.J.A. Dhert**, C.P.A.T. Klein, J.A. Jansen, E.A. van der Velde, R.C. Vriesde, P.M. Rozing, K. de Groot, "A histological and histomorphometrical investigation of fluorapatite, magnesiumwhitlockite, and hydroxylapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 27, 127-138 (1993).
25. **J.A. Jansen**, J.P.C.M. van der Waerden, J.G.C. Wolke, "Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbits", J. Biomed. Mater Res., 27, 603-610 (1993).
26. **W.J.A. Dhert**, C.P.A.T. Klein, J.G.C. Wolke, E.A. van der Velde, K. de Groot, P.M.Rozing, "A mechanical investigation of fluorapatite, magnesiumwhitlockite and hydroxylapatite plasma-sprayed coatings in goats", J. Biomed. Mater. Res., 25, 1183-1200 (1991).

Chapter 9

GENERAL DISCUSSION

The results of the investigations described in this thesis will be discussed in this chapter. Finally, based on these conclusions some recommendations for further research will be made.

9.1. OVERALL CONCLUSIONS

- This study demonstrated again the use of goats for the biocompatibility testing on the secondary level of implant materials, especially when these tests are focused on the short and prolonged behavior in the femoral trabecular bone. In addition, our findings showed the functionality of goats for evaluating specific oral implant designs. These tests require that the implants are placed in the jaws of the experimental animal, penetrate the oral mucosa and are exposed to masticatory forces. Considering these requirements and ethical criteria, mostly dogs are selected for this kind of usage tests. However, because of anatomical restrictions, dogs cannot be used to evaluate maxillary implants. In chapter 3 we found that the goat serves well the above mentioned criteria. Although, it has to be noted that important differences exist in the masticatory function of goats compared to humans, still we suppose that the obtained results can be reliably extrapolated to human situation. Certainly, since the evoked occlusal loading forces in ruminants are higher [1].
  In chapter 4 we found that beside histological and histomorphometrical evaluation methods Dual Energy X-ray Absorptiometry (DEXA) seems to be an efficient technique to predict and evaluate the bone behavior around implants. On the other hand, currently, technical restrictions hamper the use of this technique for the clinical follow up of bone mineral density around implants. First some modifications, both in hardware and software, have to be made allowing a relatively easy and accurate use.
  From a clinical point of view the most interesting finding was that, compared with as-machined titanium implants, plasma-sprayed Ca-P coatings indeed resulted in an improved response of low density trabecular bone. In addition,

we found that not only the overall bone response was increased, but also that the healing time before loading is faster when using Ca-P coatings. On the basis of these findings, we conclude that there is no need to wait longer than three months before loading implants installed in poorly mineralized bone when they are provided with a plasma-sprayed Ca-P layer. Despite this favorable conclusions, some critical remarks have to be made. For example, this study could not definitely prove that the Ca-P coating was the only responsible parameter for the improved bone behavior. We believe that the difference in surface topography between the non-coated and coated implants can have been an additional factor. We also observed that the deposited coatings dissolved or disappeared after installation. Although, we did not observe any adverse effect in bone reaction after coating loss, the effect for the long-term clinical performance of the implant is not clear. Apparently the use of higher crystalline Ca-P coatings is no solution for this problem. Recently, Clemens *et al.* [2] observed an almost complete delamination of plasma-sprayed FA coatings after long survival times (10 and 22 months). On the other hand, the above mentioned considerations plead in favor of the suggestion of de Groot [3] that apatite coatings have to be maintained for short time periods in order to benefit only the initial bone healing response. As soon as a good bone contact is obtained, the coating may gradually resorb.

9.2. FUTURE EXPECTATIONS

Considering the discrepancy between our results and the currently existing concerns about the viable use of and prognosis of plasma-sprayed Ca-P-coated oral implants [4-7], further research should concentrate on:

1. long-term animal and clinical trials to investigate the final consequences of the dissolution and delamination of Ca-P coating for their clinical applicability.

2. the development of alternative techniques for the deposition of Ca-P coatings. In this respect, RF magnetron sputter coating [8] appears to be the best choice for the future.

REFERENCES

1. **J.E. Lemons**, "Experimental approaches to tooth and bone replacement.", in: "Animal models in dental research.", ed. J.M. Navior, the University of Alabama Press, Alobama, USA, 363-389 (1977).
2. **J.A.M. Clemens**, "Fluorapatite coatings for the osseointegration of orthopaedic implants.", Phd thesis Leiden (1995).
3. **K. de Groot**, J.G.C. Wolke, J.A. Jansen, "State of the art: hydroxylapatite coatings for dental implants." J. Oral Impl., 20(3), 232-234 (1994).
4. **R. Meffert**, B. Langer, M.E. Fritz, "Dental Implants: a review.", J Periodontol., 63, 859-870 (1992).
5. **R.M. Pilliar**, D.A. Deporter, P.A. Watson, M. Pharoah, M. Chipman, N. Valiquette, S. Carter, K. de Groot, "The effect of partial coating with hydroxyapatite on bone remodeling in relation to porous-coated titanium alloy dental implants in the dog." J. Dent. Res., 70(10), 1338-1345 (1991).
6. **R.M. Pilliar**, D.A. Deporter, P.A. Watson, N. Valiquette, "Dental implant design-Effect on bone remodeling.", J. Biomed. Mater. Res., 25, 467-483 (1991).
7. **H. Vaillancourt**, R.M. Pilliar, D. McCammond, "Finite element analysis of crestal bone loss around porous-coated dental implants.", J. Appl. Biomater., 6, 267-282 (1995).
8. **J.A. Jansen**, J.G.C. Wolke, S. Swann, J.P.C.M. van der Waerden, K. de Groot, "Application of magnetron sputtering for producing ceramic coatings on implant materials.", Clin. J. Oral Impl. Res., 4(1): 28-34 (1993).

Chapter 10

SUMMARY / SAMENVATTING

Summary

Calcium-phosphate (Ca-P) ceramics have already been clinically used as implants for hard tissue replacement for more than 20 years. Hydroxyapatite has received the most attention for implantation purposes in dentistry and orthopedics. Considering the mechanical properties of ceramics, for clinical application HA has to be used as a coating on a metallic surface. Currently, the plasma-spraying process has received the most interest to deposit Ca-P coatings on medical and oral implants.

The experiments as described in this thesis were aimed to learn whether plasma-sprayed Ca-P coatings can improve the bone healing to oral implants inserted in bone of low quality. The studies were performed in an animal experimental set-up.

In **Chapter 1** the histology and bone healing processes which take place at the implant-bone interface are described. Furthermore, several factors influencing the remodeling of bone to oral implants, such as state of the host bed, implant surgery, biomechanical conditions, and implant material and surface properties, are discussed. The primary aim of the present thesis was focused on the trabecular bone response of non-coated and different plasma-sprayed Ca-P-coated titanium oral implants in order to determine whether these coatings can improve the clinical success rates and reduce the required initial healing time of oral implants before loading.

For comparative reasons there is always a need of a correct study design and evaluation criteria. In **Chapter 2** a review is presented about the required surgical, statistical and histological methods for an objective and standardized testing and evaluation of the biocompatibility of Ca-P-coated oral implants. This chapter is also an introduction to the applied experimental design and evaluation criteria of the following chapters of the present thesis.

In **Chapter 3** the evaluation of cylindrical titanium (Ti6Al4V) implants non-coated as well as plasma-spray coated with fluorapatite (FA), hydroxyapatite (HA), and heat-treated (HAHT) hydroxyapatite is described 12 weeks after installation in the trabecular bone of the lateral and medial femoral condyles of the goat. Histomorphometrical evaluation demonstrated that the coated implants showed a significant higher percentage of bone in close contact with the implant surface compared to the non-coated implants. Further, all coatings showed a non-uniform reduction in coating thickness. However, the bone-implant contact was not influenced by this coating reduction. Evaluation of three representative sections taken at different levels from the same implant revealed a significant variance in trabecular bone response. This stresses the importance not solely to rely on one section per implant. This study also proved the need of proper statistical implantation schedules before starting bone-biocompatibility tests, as a difference in bone apposition could be observed between implants in lateral and medial condyles. On the other hand, it has to be emphasized that this finding confirms the difference in bone turn-over and original bone mineral density between the lateral and medial condyles.

Therefore, Dual Energy X-ray Absorptiometry (DEXA) appears to be an excellent tool to measure the bone mineral density around implants and thus to analyze the bone-implant reaction. This method is described in **Chapter 4** by using the same experimental design as used in chapter 3. As found in the former study, regions located in the medial condyle showed a higher bone density than the lateral condylar regions. Although the histology of the implants in the medial condyle demonstrated more bone contact with coated than with non-coated implants, a higher density was measured around the non-coated implants.

The study in **Chapter 5** dealt with a comparison of cylindrical implants with the same surface characteristics as in the previous experiments, installed in the

trabecular bone of the mandibular corner of goats, which is described as a mainly unloaded implant location. However, histological evaluation showed that the mandibular corner is a dissatisfying model to install implants because of anatomical restrictions. Further, in contrast with our previous experiment performed in loaded conditions in the femoral condyles, no significant differences in bone reaction between the coated and non-coated implants could be found. The experiment remained inconclusive about the influence of loading conditions on the bone behavior, probably because of the morphological differences in bone structure between various skeletal parts.

Chapter 6 dealt with non-coated and Ca-P-coated permucosal implants installed in the maxilla. In this study the same Ca-P coatings were used as in the previous experiments. Six months after installation of the implants, they were provided with permucosal abutments. Four months later, the animals were killed. It appeared, that significantly more non-coated than Ca-P-coated implants had failed. Further, histomorphometrical analysis demonstrated an improved bone reaction to the coated compared with the non-coated implants, despite reduction in thickness of all coatings. Therefore, it was concluded that the application of Ca-P coatings can be of benefice for the bone-implant reaction in less mineralized trabecular bone.

The objective of the study as described in **Chapter 7**, was to analyze the efficacy and correlation between clinical and histological parameters used to evaluate oral implants. For this purpose, radiographs and Periotest® scores of the implants were made before histological preparation.. Results demonstrated that the histomorphometrical measurements rated the bone level more apically compared to the radiologically determined bone level. No correlation was found between the Periotest® values and bone response. Hence, we recommended a combination of various appropriate clinical evaluation techniques for the clinical follow up of oral implants as each of the techniques has its own limitations.

Finally, in **Chapter 8** the initial response of the maxillary trabecular bone to oral implants was investigated. Non-coated and Ca-P-coated threaded titanium implants were installed for 3 and 6 months. Histological evaluation revealed no difference in bone reaction between both implantation periods. Again all coatings showed reduction in coating thickness. We concluded that there is no need to wait for longer than 3 months before loading maxillary implants when they are provided with a plasma-sprayed Ca-P coating.

Chapter 9 comprises the overall conclusions. In addition some clinical implications and expectations for further research are formulated.

Samenvatting

Calciumfosfaat keramische materialen worden reeds meer dan 20 jaren gebruikt als implantatiematerialen ter vervanging van bot. Voor tandheelkundige en orthopedische toepassingen wordt meestal gebruik gemaakt van hydroxyapatiet (HA). Omwille echter van de ontoereikende mechanische eigenschappen van keramische materialen wordt HA als een coating aangebracht op het metalen implantaat oppervlak. De meest gangbare procedure om coatings aan te brengen op implantaten is het plasma-sprayen. Het doel van dit proefschrift is om bij middel van proefdierexperimenten na te gaan of calciumfosfaatcoatings die via een plasma-spray procedure werden aangebracht op tandheelkundige implantaten de heling van bot van lage densiteit kan verbeteren.

Het **eerste hoofdstuk** beschrijft de histologie en helingsprocessen van het bot ter hoogte van het grensvlak tussen implantaat en bot. Ook andere factoren die een invloed kunnen hebben op de hermodellering van het bot worden besproken, zoals de toestand van het bot waarin de implantaten worden geplaatst, de chirurgische procedure, biomechanische factoren, het implantaat materiaal, en de oppervlakte eigenschappen van het gebruikte materiaal. In dit proefschrift werd voornamelijk de invloed van de trabeculaire botreactie van ongecoate en verscheidene Ca-P gecoate titanium implantaten nagegaan om te bepalen of deze coatings het klinisch success kunnen verbeteren en aldus ook de inhelingsperiode, alvorens implantaten te belasten, kunnen verkorten. Wil men studies onderling kunnen vergelijken moet men de studie op een juiste manier opzetten en de juiste evaluatie criteria hanteren. **Hoofdstuk 2** geeft een overzicht van de chirurgische, statistische en histologische criteria die vereist zijn om te komen tot een objectief en gestandaardizeerd onderzoek voor de evaluatie van de biocompatibiliteit van Ca-P gecoate tandimplantaten. Dit hoofdstuk is ook een inleiding tot de in de volgende hoofdstukken van deze thesis gebruikte experimentele studieopzet en evaluatie criteria.

Het **derde hoofdstuk** beschrijft de evaluatie van cilindrische titanium (Ti6Al4V) implantaten, ongecoat dan wel gecoat met fluorapatiet (FA), hydroxyapatiet (HA), en hitte behandelde hydroxyapatiet (HAHT). Deze implantaten werden aangebracht in het trabeculaire bot van de mediale en laterale femorale condylus van de geit.
De histomorphometrische evaluatie toonde een significant hoger percentage botcontact met de gecoate implantaten in vergelijking met de ongecoate implantaten. Alle coatings vertoonden een reductie in dikte wat echter de botreactie niet beïnvloedde. Een significante variatie werd waargenomen in de trabeculaire botrespons bij het evalueren van drie representatieve histologische coupes van eenzelfde implantaat op verschillende niveaus. Het is daarom van belang meer dan één coupe per implantaat te beoordelen. Daar we ook een verschil in botappositie zagen tussen implantaten die aangebracht werden in de laterale en mediale condylus, toont deze studie ook aan dat men moet vertrekken van correcte statistische implantatie schema's. Van de andere kant moet worden benadrukt dat deze resultaten het verschil bevestigen in bot hermodellering en oorspronkelijke bot minerale dichtheid tussen de laterale en mediale condylus.

Daarom blijkt de Dual X-ray Absorptiometry (DEXA) een goede methode te zijn om de minerale dichtheid van bot op te meten en aldus de bot-implantaat reactie te analyzeren. In **hoofdstuk 4** wordt deze methode beschreven door gebruik te maken van hetzelfde proefopzet als in hoofdstuk drie werd gebruikt. De regio's die zich in de mediale condylus bevonden vertoonden een hogere minerale botdichtheid dan de laterale regio's. Ondanks histologisch onderzoek meer bot in contact met de gecoate implantaten dan met de ongecoate implantaten in de mediale condylus aantoonde, werd er een hogere minerale botdichtheid opgemeten rond de ongecoate implantaten.

In **hoofdstuk 5** wordt een vergelijking gemaakt tussen cilindrische implantaten met dezelfde oppervlakte eigenschappen als de implantaten gebruikt in de

vorige experimenten. De implantaten werden aangebracht in het trabeculaire bot van de kaakhoek van de mandibula van de geit, daar deze regio in de literatuur beschreven wordt als zijnde onbelast. Histologische evaluatie toonde echter aan dat deze locatie een ontoereikend model is om implantaten aan te brengen omwille van anatomische beperkingen. Verder vonden we in dit onbelaste model geen significante verschillen in de botreactie tussen de gecoate en ongecoate implantaten, in tegenstelling tot de vorige studies die onder belaste voorwaarden plaatsvonden. Een besluit over de invloed van belasting op de botrespons kan men aan de hand van deze studie niet trekken, waarschijnlijk omwille van morfologische verschillen in de structuur van het bot tussen de mandibula en de femur.

Hoofdstuk 6 belicht ongecoate en calciumfosfaat gecoate permucosale implantaten die aangebracht werden in de maxilla van de geit. Dezelfde coatings werden gebruikt in deze studie als in de vorige experimenten. Zes maanden na het plaatsen van de implantaten werden deze geconnecteerd met de permucosale abutments. Vier maanden later werden de dieren opgeofferd. Een significant hoger aantal van de ongecoate implantaten dan van de gecoate implantaten ging verloren. Ondanks de reductie in coating dikte van alle coatings, toonde histomorphometrisch onderzoek een betere botreactie aan ter hoogte van de gecoate implantaten in vergelijking met de ongecoate implantaten. Daarom kunnen we uit deze studie besluiten dat het gebruik van calciumfosfaat coatings op implantaten die aangebracht worden in bot van lage dichtheid, de botrespons gunstig beïnvloeden.

In **hoofdstuk 7** belichten we de relatie tussen klinische en histologische parameters om tandimplantaten te evalueren. Naast de histologische preparatie van de implantaten die in de studie van hoofdstuk zes werden gebruikt, werden op voorhand röntgenfoto's genomen en mobiliteitsmetingen van de implantaten uitgevoerd. Aan de hand van de histomorphometrische metingen bleek het botniveau meer apicaalwaarts te liggen in vergelijking met

het röntgenologisch opgemeten botniveau. Er werd geen verband gevonden tussen de Periotest® metingen en de botreactie. Het is daarom aan te bevelen steeds een combinatie van evaluatie technieken te gebruiken in de klinische follow-up van tandimplantaten, daar elke methode beperkingen heeft.

In **hoofdstuk 8** werd de initiële botreactie nagegaan van gecoate en ongecoate schroefvormige implantaten die geplaatst werden in het trabeculaire bot van de maxilla van de geit. Er werd geen verschil gevonden in de botrespons tussen de 3 en 6 maanden implantatie periode. Opnieuw vertoonden alle coatings een afname in dikte. Aan de hand van deze studie kunnen we besluiten dat het niet nodig is om langer dan drie maanden te wachten met het belasten van implantaten in de maxilla wanneer deze voorzien zijn van een plasma-spray calciumfosfaat coating.

In **hoofdstuk 9** worden de bevindingen van dit proefschrift besproken en met elkaar in verband gebracht. Enkele klinische implicaties en raadgevingen voor verder onderzoek worden tot slot geformuleerd.

ACKNOWLEDGEMENTS

This thesis is a result of cooperation between the Department of Prosthetic Dentistry, BIOMAT research group, of the University of Leuven, Belgium and the Department of Oral Function, Laboratory of Biomaterials of the University of Nijmegen, The Netherlands.

I want to express my gratitude to Prof. R. Dillemans, former rector of the Catholic University of Leuven, and to Prof. A. Oosterlinck, present rector of the Catholic University of Leuven for giving me the opportunity to undertake this study.
I would like to express my sincere gratitude to all of you that have helped me during the work with this thesis. Especially, I would like to thank for their help, advice or assistance:
Prof. I. Naert, head of the Department of Prosthetic Dentistry of the Catholic University of Leuven, for giving me the opportunity and the facilities to undertake this research. Despite his time-consuming clinical and experimental work, he always made time for the many helpful discussions when analyzing the results and was a critical reviewer of the articles that made part of the present thesis.
Prof. J.A. Jansen, head of the Laboratory of Biomaterials of the Department of Oral Function of the Catholic University of Nijmegen, for introducing me into this field of research, for his constant support, guidance and never failing enthusiasm and for his special way of creating a friendly and creative atmosphere.
Prof. W. Kalk, head of the Department of Oral Function of the Catholic University of Nijmegen for his critical review of this thesis and his confidence in my work.
J.P.C.M. van der Waerden who cooperated with enthusiasm in helping me to prepare the histological sections, the histomorphometry, the photographs and drawings in this thesis. He was one of the persons I could rely on when my time planning was too tight again.
Drs. A.G. Hulshof, it was nice to have her at the desk next to mine; we shared joys and sorrows, but there was also a fruitful cooperation with lines for discussions and criticism.
Many others were of great help during the last years and in particular the following persons are gratefully acknowledged:
Mrs. F. Mattheus and Mr. J. Geukens for their help in solving many technical and computer problems. The personnel of the animalium of Nijmegen for their help, training, support and care for the animals. My colleagues from the Laboratory of Biomaterials; Joop, Kitty, Edwin, Yvonne, Anja, Suzy, Gonny, Piet, Harry, Hila, Anneke, Tohru and Frans for being true colleagues and making my stay at The Netherlands a very enjoyable one.

Finally, I want to thank my husband Bart for his stimulating attitude and patience.

CURRICULUM VITAE

The author was born on August 1st, 1968 in Herk-de Stad. After graduating from high school (ASO, Ursula-instituut te Herk-de-Stad), she started in 1986 her study in dentistry at the University of Diepenbeek (LUC). She obtained her degree in dentistry in June 1991 at the Catholic University of Leuven. Thereafter she started a post-graduate program at the Department of Prosthetic Dentistry of the Catholic University of Leuven (Head: Prof. Dr. I. Naert). From June 1992 until June 1996 she also worked part-time as a research fellow at the Laboratory of Biomaterials of the Department of Oral Function of the University of Nijmegen (Head: Prof. Dr. J.A. Jansen). From July 1996 she continues her post-graduate program in prosthetic dentistry at the Catholic University of Leuven.

ACTA BIOMEDICA LOVANIENSIA

1. P. AGOSTINIS, Protein Phosphatases. Substrate specificity and enzyme regulation by second-site phosphorylation. 1988
2. L. MISSIAEN, Characterisation and regulation of the Mg^{2+}-ATPase and the (Ca^{2+}+Mg^{2+})-ATPase in smooth-muscle microsomes and its possible relevance for smooth-muscle function. 1988
3. Y. VANRENTERGHEM, Nonsteroidal Anti-inflammatory Drugs in the Treatment of Chronic Glomerulonephritis. A clinical and experimental study. 1988
4. B. HIMPENS, Free Intracellular Calcium during smooth Muscle Excitation. 1988
5. E. WAELKENS, Regulation and Specificity of the Polycation-stimulated Protein Phosphatases. 1988
6. B. GULYAS, Relative Motion Sensitivity in the Visual System of Cats and Monkeys. 1988
7. J. ANDRES, The Postischemic Myocardium. Energetic State and its Relation to Contractile Failure. 1988
8. J. VAN ELDERE, Intestinal Steroid Desulfation. Isolation and Characterization of Intestinal Steroid Desulfating Bacteria and their Influence on the Enterohepatic Circulation of Steroids. 1988
9. ILONDO MBELENGE MAPOKO, Cellular Receptors for Human Growth Hormone. Quantitative Aspects and Clinical Applications. 1989
10. P. CARMELIET, Production, Release and Paracrine Action of Acetylcholine in the Anterior Pituitary of the Rat. 1989
11. KABI MUKUNYA, Hémorragie Périventriculaire et ntra-ventriculaire du Nouveau-né. Etude Clinique et Ultrasonographique. 1989
12. W. ALLAERTS, Involvement of Folliculo-stellate Cells in Inhibitory Interactions in Rat Anterior Pituitary. A Morpho-functional Study *in Vitro*. 1989
13. C. ROELANT, A comparative Study of Luminol and Lucigenin in the Mediation of Chemiluminescence produced by Stimulated Human Polymorphonuclear Neutrophils and Neutroplasts. 1989
14. D. DECLERCK, Dental Extractions and Compromised Hemostatis. An experimental and clinical study. 1989
15. A. RÓNASZÉKI, Hemodynamic Consequences of the Timing of Atrial Contraction during Complete Atrioventricular Block. 1989
16. K. HEMSCHOOTE, Characterization of an Androgen-Regulated mRNA Encoding Multiple Proline-Rich Polypeptides in the Rat Ventral Prostate. 1989
17. F. CLAESSENS, Study of the Androgen Regulated Genes of Prostatic Binding Protein Functional Characterization of the C3(1) Intronic Androgen Response Element. 1989
18. P. HERDEWIJN, Dideoxynucleoside Analogues as Inhibitors of the Replication of Human Immunodeficiency Virus (HIV). 1989
19. X. CAYLA, Protein Phosphatases of *Xenopus Oocytes*. Phosphotyrosyl phosphatase activation of the PCS phosphatases. 1990
20. C. SMETS, A Knowledge-based System for the Automatic Interpretation of Blood Vessels on Angiograms. 1990
21. R. ROELANDTS, Analyzing the Photochemotherapeutical Principle in the Treatment of Psoriasis. 1990
22. J. VAN AERDE, Intravenous Nutritional Energy Support and Macronutrient Utilisation in the Neonate. 1990

23. F. DE ZEGHER, In Vivo Studies on Growth Hormone Secretion in the Human and Ovine Fetus and Newborn. 1990
24. A. VANDEKERCKHOVE, Studies on Receptors involved in Liver Glycogenolysis in Rabbit, Guinea Pig and Man. 1990
25. L. MORTELMANS, Scintigraphic Evaluation of Coronary Thrombolysis. An experimental and clinical study. 1990
26. W. ROBBERECHT, Control of Growth Hormone and Prolactin Release by Angiotensin Peptides in Reaggregate Cell Cultures of the Rat Pituitary.
27. M. JORISSEN, Ciliogenesis and Ion Channels in Human Nasal Epithelial Cells Cultured in Vitro. 1990
28. G. F.G. MOENS, Aspects of the Epidemiology and Prevention of Suicide. 1990
29. J. TYTGAT, Comparison between Cardiac L- and T-type Calcium Channels: an Electro- Physiological and Pharmacological Study. 1990
30. L. VAN DEN BOSCH, Characteristics and Development of the Na^+-Dependent Hexose Carrier Expressed in Renal Established Cell Lines. 1990
31. M. CASTEELS, Differences in the Peroxisomal ß-Oxidation of Fatty Acids and Bile Acid Intermediates. 1990
32. E. BIELEN, The Inheritance of Cardiac Structure and Function at Rest and During Dynamic Exercise: an Echocardiographic Study in Twins. 1990
33. J. EGGERMONT, Molecular Characterization of the CA^{2+}-pump Isoforms in the Endoplasmic Reticulum of Smooth Muscle. 1990
34. C. VAN GEET, Signal Transduction in Human Endothelial Cells and Endothelial Cell and Platelet Activation in Vivo in Man. 1990
35. L. SCHEPERS, Peroxisomal ß-Oxidation of the Carboxyl Side Chains of Prostaglandins and Bile Acid Intermediates. 1990
36. I. DECLERCK, Modulation of the Ca^{2+} movements in Vascular Smooth Muscle Cells by the Co-transmitters Noradrenaline and ATP. 1991
37. B. DE STROOPER, Biosynthesis and Expression of the Human $\alpha_5\beta_1$-Integrin Fibronectin Receptor. 1991
38. M. GEWILLIG, Cardiac and Circulatory Adaptation after the Fontan Operation. 1991
39. T. BRZOSTEK, Thrombolysis and Acute Myocardial Infarction: Early and Late Effects on Clinical Status, Left Ventricular Function and Exercise Capacity. 1991
40. I. E. NAERT, The Influence of Prosthetic Design and Implant Type on Tissue Reactions around Oral Implants. 1991
41. A. HEREMANS, Matrix Heparan Sulphate Proteoglycan: Structure, Assembly and Core Protein Binding Interactions. 1991
42. F. BIELEN, Sodium-proton Exchange in Cardiac Cells: Role in Modulation of Intracellular Sodium and Sodium-dependent Processes. 1991
43. H. HOUBEN, Study on the Presence of Bombesin-like Peptides and their Effects on Hormone Release and Cell Multiplication in the Rat Anterior Pituitary. 1991
44. I. TORNAI, New Aspects of Platelet-Von Willebrand Factor Interaction. 1991
45. K. LEE, The Plasma Native Alkaline Phosphatase is a Phosphotyrosyl Phosphatase. 1991
46. H. HEIDBÜCHEL, Functional Properties of Atrial Muscarinic K^+ Channels. An Electrophysiological Study in Human Guinea-pig and Frog. 1991

47. L. LAGAE, A Neurophysiological Study of Optic Flow Analysis in the Monkey Brain. 1991
48. W. SPILEERS, A Study of the Neuronal Characteristics of Pattern Reversal Visual Evoked Cortical Potentials with Special Focus on Contrast Modulated Steady State Visual Evoked Potentials (CMSS VEPs). 1991
49. M. BOLLEN, The Structure and Regulation of Type-1 Protein Phosphatases Involved in Hepatic Glycogen Metabolism. 1991
50. S. WERA, Purification and Characterization of the Glycogen-bound Protein Phosphatase from Rat Liver. 1991
51. E. VANDENBERGHE, Clinical Morphological, Immunophenotypic and Genetic Characterization of Follicle Mantle Lymphomas. 1992
52. K. DEVRIENDT, Molecular Characterization of the Human Pregnancy Zone Protein. 1992
53. J. TACK, Physiology of the Enteric Nervous System in the Gastric Antrum of the Guinea-Pig. 1992
54. D. TILEMANS, Paracrine Control of the Development of Anterior Pituitary Cells in the Rat. 1992
55. P. J. HOOFT, Seminal Zinc as a Screening Instrument for the Preliminary Identification of Semen Traces. 1992
56. K. SIPIDO, CA^{2+} Release from the Sarcoplasmic Reticulum of Cardiac Myocytes. 1992
57. P. HENDRIX, Protein Phosphatase 2A Structure and Regulation. 1992
58. E. K. VERBEKEN, An Anatomical Study of Lung Mechanics in Man. 1992
59. L. HSIANG-NING, The Na^+-Activated K^+ Channel in Cardiac Cells: Physiological and Pharmacological Characterization. 1993
60. X. BAI, Heparan Sulfate Proteoglycans in Embryonic Development. 1993
61. S. JANSSENS, Effects of Theophylline on Respiratory Skeletal Muscle: An Experimental Study in Dogs. 1993
62. G. MASSA, The Growth Hormone-Binding Proteins in Rat and Man. 1993
63. L. DESMEDT, Two Novel Cation-Selective Channels in the Apical Membrane of Frog Skin (*Rana Temporaria*): Fluctuation and Small-Signal Analysis of Inactivation by CA^{2+}. 1993
64. M. GARMYN, Responses of Mammalian Skin to Ultraviolet Irradiation: A Combined Morphological and Molecular Study. 1993
65. I. BUYSE, Association of HLA Class II Antigens with Insulin-Dependent Diabetes Mellitus. 1993
66. B. BONTE, Jaw Reflexes of Periodontal Origin. 1993
67. P. DE LEYN, Patterns and Site of Adenosine Triphosphate Catabolism in Lung Tissue. 1993
68. S. DE JAEGERE, Molecular Characterization of Plasma-Membrane Ca^{2+}-transport ATPase Isoforms from the Pig (*Sus scrofa*). 1993
69. F. TSHIPAMBA MPUILA, Biochemical Molecular and Functional Properties of the Ca^{2+} Homeostasis in Renal Cells in Culture. 1993
70. D. OOSTERLYNCK, Studies on the Natural Killer Mediated Cytotoxicity in Women with Endometriosis. 1994
71. G.M. VERLEDEN, Modulation of Neurotransmission in the Airways: an Experimental Study in Guinea-Pig and Human Airways in Vitro. 1994
72. G. CARMELIET, Modulation of β_1 Integrin Function During Neurite Outgrowth. 1994
73. F. STAMMEN, Percutaneous Transluminal Coronary Angioplasty: a clinical follow-up study. 1994
74. K. PEERLINCK, Von Willebrand's Disease and Hemophilia, Clinical and Genetic Aspects. 1994

75. G. VANHOVE, Peroxisomal ß-Oxidation of 2-Methyl-substituted Fatty Acids. 1994
76. G. VAN den BERGHE, Dopamine and Pituitary Hormones in Critical Illness. 1994
77. Z DEBYSER, Viral DNA Synthesis, A Study of Viral DNA Synthesis in Two Model Systems. HIV-1 Reverse Transcription and DNA Synthesis at the Replication Fork of Bacteriophage T7. 1994
78. X. BOSSUYT, Regulation of Hepatic Microsomal UDP-Glucuronosyltransferases and Nucleotide Sugar: Dolichyl Phosphate Glycosyltransferases. 1994
79. S. VAN LIERDE, Respiratory Distress Syndrome: Early Prediction of Outcome and Characterization of Two Types of Bronchopulmonary Dysplasia. 1994
80. M. WILLEMS, Hepatitis C, Medical Virology and Experimental Study. 1994
81. C. HILLIKER, Molecular and Genetic Characterization of Selected Loci of the A2M-system. 1994
82. L. OVERBERGH, Molecular Characterization of the Munnoglobulins. 1994
83. M HENDRIKX, Endogenous Myocardial Protection Against Ischemic/ Reperfusion Injury. 1994
84. W. MEULEMANS, The Genetics of Dizygotic Twinning. 1994
85. L. VERRESEN, Bradykinin: a New Marker of Bio(in)Compatibility in Hemodialysis. 1994
86. J. VAN LINT, Serine/Threonine Kinases and the Signal Transduction of Mitogenic, Cytotoxic, and Chemotactic Factors. 1994
87. C. VAN HOOF, PTPA, A Protein Controlling the Dual Specificity of Protein Phosphatase 2A. 1994
88. W DESMET, Restenosis after percutaneous Transluminal Coronary Angioplasty: Detection, Assessment and Modes of Prevention. 1994
89. M. HERREGODS, Echocardiography in Ischemic Heart Disease. 1994
90. M. BOSCH, Protein Phosphatase Type-2A in *Xenopus Laevis*. 1994
91. M. DEPRÉ, Leukotrienes, Development of Assay Methodology in Biologic Fluids and Evaluation of Antagonists *in vitro* and *in vivo* in Man. 1994
92. J. ARNOUT, The Antiphospholipid Syndrome. 1994
93. G. MERTENS, Cell Surface-Associated Heparan Sulfate Proteoglycans, Binding Properties and Subcellular Expression. 1994
94 D VAN GOOL, Studies in Pathogenesis of Brain Lesions in Alzheimer's Disease and Related Disorders. 1994
95 M. URIARTE, Phosphorylation and Dephosphorylation of the Ecto-Nucleotide Pyrophosphatase *PC-1* from Rat Liver 1994
96. M ZAMANI GHABANBASANI, Association Studies between HLA Class II, CD3 and CD4 Polymorphisms and Diabetes, Multiple Sclerosis and Schizophrenia. 1994
97 M. VERHAEGEN, A Comparison of Halothane and Isoflurane on Celebral Ischemic Thresholds as Assessed by EEG and Cortical Depolarization. 1994
98 R. DEKHUIJZEN, Effects of Glucocorticosteroids on Rat Diaphragm: A Physiological and Histopathological Study. 1994
99. P. DE COCK, Ontwikkelingsperspectieven bij kinderen met geboortegewicht beneden 2000 G 1994

100. R. MAEX, Direction-Selective Simple Cells in Cat Striate Cortex: A modeling study. 1994
101. M. ZIZI, VDAC, a Mitochondrial Outer Membrane Channel: Physiological Regulation and Biophysical Properties. 1994
102. P. BROCK, Cisplatin Toxicity in Infants and Children. 1994
103. P. WOUTERS, Functional Adaptation to Myocardial Ischemia: The Relationship between Regional Contractile Function and Perfusion in Conscious Dogs. 1994
104. M. MISEREZ, Characteristics of Contractile Dysfunction in Human Heart Failure. 1994
105. R. DECORTE, Application of the Polymerase Chain Reaction to Forensic DNA Analysis in Belgium. 1994
106. H. VERBOOMEN, The SERCA2-Type Ca^{2+}-Transport ATPases in the Pig *(Sus scrofa)*. Isoform Diversity and Regulation by Phospholamban. 1995
107. F.R. WALDENBERGER, Novel Cardiac Assist Devices with Different Unloading Capacities. 1995
108. H. CLAES, Research on Venous Outflow Reduction in Erectile Dysfunction. 1995
109. C. GUO, The Molecular Genetics of X-Linked Alport Syndrome. 1995
110. H. CUPPENS, Genetic Studies of the Cystic Fibrosis Transmembrane Conductance Regulator Gene in Belgian CF Patients. 1995
111. M. BAENS, Isolation and Regional Assignment of New Genes on the Short Arm of Human Chromosome 12. 1995
112. P. DEMAEREL, Clinical Applications of Localized ^{1}H-NMR Spectroscopy in Intracranial Tumours. 1995
113. J. N. HAN, Breathing Patterns in Anxiety Disorders: Is there a Hyperventilation syndrome? 1995
114. S. AZIZ, On the Nature of the Bilirubin Pigments in the Newborn Infant. 1995
115. M. DE HERT, Suïcide bij jonge schizofrene patiënten. 1995
116. R. DERUA, Characterization and Physiological Importance of a Novel Cyclin-B2-Kinase in *Xenopus laevis*. 1995
117. I. ZINK, De voorspelbaarheid van de relatieve taalvaardigheid bij jonge, normaal ontwikkelende kinderen: een follow-up onderzoek aan de hand van de RTOS. 1995
118. P. THEYS, Perceptie van de kwaliteit van slaap en waaktoestand in psychofysiologische insomnia. 1995
119. A. VAN EYNDE, Characterization and Molecular Cloning of NIPP-1, a Novel Regulatory Subunit of Protein Phosphatase-1. 1995
120. L. MERTENS, Regulation of Alternative Processing of the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase Gene 2 Transcript during Muscle Differentiation. 1995
121. M. FRANSEN, Peroxisome Biogenesis: Identification and Characterization of the Import Receptor for Peroxisomal Proteins Containing a C-Terminal Targeting Sequence. 1996
122. D. MOECHARS, A Transgenic Mouse Model for Alzheimer's Disease. 1996
123. L. UMANS, Targeted Inactivation («knock-out») of the Mouse Genes coding for Alpha-2-Macroglobulin and for the A2M-Receptor Associated Protein. 1996
124. M. GERICKE, Calcium Entry in Endothelium and in Mast Cells - Mechanisms and Modulation. 1996

125. R. Wu, Molecular Genetics of Type 1 Neurofibromatosis - Towards preimplantation genetic diagnosis. 1996
126. C. M. L. Bollen, Full-mouth Disinfection and Hard Surface Smoothening Reduce the Subgingival Microbial Load. 1996
127. A. Van Bael, Paracrine Growth Factors Control the Development of Lactotrophs and Somatotrophs. 1996
128. B. Nuttin, Interleukine-6 en beschadiging van het ruggemerg bij de rat. 1996
129. B. Coulie, Role of 5-Hydroxytryptamine-1 Receptors in the Regulation of Gastrointestinal Motility. 1996
130. J. Aerssens, Molecular Analysis of a Chromosomal Translocation Breakpoint on the Short Arm of Chromosome 12. 1996
131. A. Maes, Regional Myocardial Blood Flow and Metabolism in Chronic and Acute Heart Disease: a Study with Positron Emission Tomography. 1996
132. L. Van Rompaey, Recombinant Human α-Macroglobulins. Analysis of Two Functional Domains: the Internal Thiol Ester and the Bait Domain. 1996
133. S. Vanderschueren, Thrombolytic Properties of Recombinant Staphylokinase in Experimental Animal Models and Clinical Trials. 1996
134. R. Steinfeld, Modulation of the Interaction between Fibroblast Growth Factor-2 and Fibroblast Growth Factor Receptor-1 by Cell Surface Heparan Sulfate Proteoglycans. 1996
135. A. D'Hulst, Linear Carbohydrates as Chiral Selectors in Capillary Electrophoresis. 1996
136. H. Caulier, An Animal Experimental Study to Improve the Success Rate of Oral Implants in Bone of Low Density: The Influence of Ca-P Coatings. 1996